1948

ANNUAL HISTORICAL REPORT

U.S. Army.

406TH MEDICAL GENERAL LABORATORY

TOKYO, HONSHU, JAPAN

(SUBMITTED IN COMPLIANCE WITH AR 40-1005)

"It is a truism of science that a study of rare and curious events in nature often brings to light general phenomena or principles which may be exaggerated in the rare but overlooked in the commonplace." Henrici, 1940

FOREWORD

In the early phases of the occupation four medical laboratories (Army) were brought into Japan. Discussion concerning the establishment of a central laboratory in Japan was initiated by a letter (9 January 1946) from Brigadier General James S. Simmons, then Chief of Preventative Medicine Section of the Surgeon General's Office to Brigadier General Joseph I. Martin, then Chief Surgeon, AFPAC. In this letter General Simmons indicated that the Surgeon General considered that the establishment of such a unit was desirable but indicated that personnel would be a definite problem. Following this there were a series of local discussions, eventuating in activation of the 406th Medical General Laboratory on 10 May 1946 by Letter Order Number 5-16, Headquarters Eighth Army, dated 7 May 1946, under T/O & E 8-500, Column HA, dated 18 January 1945. The unit was temporarily housed in Yokohama while laboratory quarters w ere prepared in Tokyo.

On 30 September 1946 the unit moved to the Mitsubishi Higashi Building Number 7 in the Maranouchi District in downtown Tokyo. Previous reports have covered fully the initial problems encountered in the development of a new organization and a new physical plant in an occupied area.

During 1947 the staff increased in size as did the variety and magnitude of the work encompassed. From a unit carrying mainly routine tasks, the development of various investigations and epidemiological studies dealing with diseases indigenous to this area occupied most of 1947. At the end of 1947 the staff consisted of twenty-two officers, fourteen Department of the Army civilians, sixty-eight enlisted men, ten foreign nationals, and ninety-eight Japanese nationals.

PREFACE

The mission of the 406th Medical General Laboratory is "to supplement the epidemiologic, sanitary and diagnostic laboratory services available in other medical department laboratories. This function includes the investigation, by laboratory methods, of outbreaks of disease and of conditions which may or do affect the health of military personnel or animals." (Circular 106, General Headquarters, Far East Command, 14 November 1947). During 1948 the unit provided definitive laboratory service to Japan, Korea, and the Marianas-Bonins. In addition, certain special problems in the Ryukus were undertaken.

In previous reports administrative procedures were treated at some length. Since there have been but few changes during the calendar year 1948, most of the procedures described in the previous annual reports remain in effect. Consequently, a detailed discussion would be, in the main, a duplication. (The Administrative Section is included only in those reports intended for the Surgeon General and for intermediate headquarters). Again the discussion of routine laboratory procedures has been eliminated, feeling that with certain exceptions, such procedures are more or less common to all laboratories.

Portions of the report labeled "Special" or "Research" cover work accomplished during standby periods when the services of various individuals were not in immediate demand. Such a procedure serves to keep the staff gainfully occupied, and familiar with special techniques, should more immediate disease problems facing an Occupation Force in the Far East demand their special knowledge. Some of the sections of the report represent the accumulation of the work of several years. All sections deal with work in progress, much of which remains to be completed, either by additional field work, or by the application of analytical procedures to data already amassed.

Throughout the report emphasis has been placed on data of epidemiological significance and rather extensive treatment has been given to diseases peculiar to this area, or which, because of special conditions, can be more readily studied here than in other localities accessible to Medical Department activities.

The majority of the maps are based on Central Japan 1:250,000, A.M.S. L571, (AMS 2), 1945, and Northern Honshu, Central Honshu, Southern Honshu, and Kyushu 1:50,000, A.M.S. L775, (AMS 1), 1944. Place names are transcribed according to the Modified Hepburn System. With but an occasional exception all of the graphic presentations in the report were prepared by the Statistics and Reports Section of GHQ.

In addition to aid generously given by all echelons of medical command of both Army and Navy, it is desired to acknowledge particularly the cooperation and assistance of the Public Health and Welfare Section of the Supreme Command for the Allied Powers, Eighth Army Military Government, many of the Prefectural Military Governments (in Tokyo, Okayama, and Yamanashi this support has been practically continous), the Third Military Railway Service, the Photographic Division of the Signal Section of GHQ, the Allied Translator Interpreter Service of the G-2 Section of GHQ, the Statistics Branch of the Natural Resources Section of GHQ, the 64th Engineer Topographical Battalion, the Military Government of the Ryukus, and the Naval Government of Guam.

Invaluable assistance has been given by members of the Commission on Virus and Rickettsial Diseases of the Army Epidemiological Board. Finally, even brief perusal of the report will indicate the part played by the Army Medical Department Research and Graduate School, Washington, since the well-known short title "AMDR&GS" appears with regularity.

As in the past, this report has been compiled by the officers or civilians in charge of the various laboratory and administrative sections. It reflects the efforts of numerous individuals at all levels of the unit.

W.D.T.

CONTENTS

MEDICAL ZOOLOGY

The work accomplished by the Section of Medical Zoology during 1948 may be divided into several categories as follows: Routine stool examinations on certain groups of Japanese Nationals employed by the Army; routine stool examinations on Occupation personnel; special tests; special research projects; training activities of officers, enlisted men, and Foreign and Japanese National personnel; preservation and distribution of parasitic materials and specimens for research and teaching purposes.

A summary of all stool specimens examined during 1948 appears as Table I. The numbers represent specimens submitted and are not to be confused with the number of individuals examined.

Table I. Summary of Stool Specimens Examined During 1948

Routine Stool Examinations (Japanese)	3255
Routine Stool Examinations (Occupation)	4760
Stools Examined During Drug Assay	1188
Stools Examined During Prefectural Surveys	
Yamanashi	2550
Okayama	1263
Hiroshima	809
Saga-Fukuoka	716
Kumamoto	523
Kagoshima	405
Ohita	429
Chiba	840
Ibaraki	1065
Saitama	620
Tokyo	422
South Korea	915
Total	19,760

Routine

Table II. Incidence of Parasitism in Occupation Personnel and Japanese Nationals

	Occupation Personnel[x]		Japanese Nationals	
	Number	Percent Infected	Number	Percent Infected
No. persons examined	3957		3077	
No. parasitized	1390	35.2	2287	74.3
No. with helminths	701	17.7	1974	64.2
No. with protozoa	951	24.0	842	27.4
Helminths:				
Ascaris	377	9.5	1429	46.4
Whipworm	202	5.1	704	22.9
Hookworm	107	2.7	408	13.3
Trichostrongylus sp.	57	1.4	377	12.3
Clonorchis sinensis	10	0.3	26	0.8
Hymenolepis nana	3	0.1	8	0.3
Strongyloides stercoralis	7	0.2		
Pinworm	6	0.2	17	0.6
Taenia sp.			1	0.03
Metagonimus yokogawai			4	0.1
Protozoa:				
Endamoeba histolytica	158	4.0	111	3.6
E. coli	474	12.0	529	17.2
Endolimax nana	407	10.3	375	12.2
Giardia lamblia	156	3.9	119	3.9
Iodamoeba butschlii	27	0.7	25	0.8
Dientamoeba fragilis	1	0.02		
Trichomonas hominis	4	0.1		
Enteromonas hominis	5	0.1		
Chilomastix mesnili	29	0.7	21	0.7

The comparative infection rates computed on the basis of stool samples submitted from Occupation and Japanese sources for routine examinations are shown in Table II. The methods of routine examination are identical to those used for survey purposes and are noted under that section of the report.

Special

A limited number of specimens have been received for the diagnosis of malaria and filariasis, and sputum for paragonimiasis. Various entomological and other specimens were submitted for identification. In some instances measures of control were sought. Material such as appendices was received from the Section of Pathology for confirmation, and/or identification, of parasites.

Two dozen South African clawed frogs (Xenopus laevis) were received late in November for use in pregnancy tests. The technic of running the test was first checked on known positives. Since beginning to run these diagnoses 12 routine tests for pregnancy or neoplasms have been completed and three were positive. The chief problem lies in the proper control of virus or bacterial diseases such as "red leg" which to date has apparently caused the death of eight frogs.

Research

Studies on Schistosomiasis - While considerable progress has been made since July 1947 this must be regarded in the nature of a progress report. It is important to know both from a military and/or a public health viewpoint how to control cercariae and the snail hosts. Information on other potential snail hosts of S. japonicum is also desirable.

(1) Studies on Population Groups and Habitats of the Intermediate Host - Observations on the habits of Oncomelania nosophora have continued in 1948 in the Yamanashi endemic area. On the 15th of each month more than 2000 snails were obtained from four collecting stations (Fig. I). The habitats varied from a high, relatively dry (Mutsusawa) to a low, very wet one (Ido). All of these snails were measured and crushed for examination.

In general the following points can be made. The adult population of O. nosophora ranged from 6 to 10 mm, with most of them between 7.5 and 8.5 mm in length. When measured to the closest 0.5 mm they usually form a unimodal population curve, in contrast to the bimodal curve typical of the closely related species O. quadrasi. The ratio of females to males, from two stations in November, was 53.2 to 46.8. The rate of infection in the two sexes was practically the same. The smaller snails in the adult population are predominantly males and the larger ones females. In the lowland areas observed the shells were soft and in one they were greatly eroded. At the dryer, upland station the shells were very hard and did now show much erosion, however crayfish, a natural control potential, were able to crush and eat them without difficulty. Young snails began to appear in the wettest station first. In June 2.2 percent of the snails at Ido belonged to the new generation. By July 73.0 percent of the population were young snails. In the driest areas the production of young was delayed about one month. In the wettest area most of the egg laying was done at one time, in the spring. In the driest habitat young snails appeared almost continuously until October. In the intermediate habitats the habits of reproduction were also intermediate and most of the young snails appeared after the ditches had been used for irrigation. Physical changes of the habitat made early in May at the Shida station almost stopped reproductive activity. There has been no evidence that the adult population, produced in the spring of 1947 or before, is dying out. The snails certainly live longer than 18 months but observations have not been continued long enough to determine how long they do live.

Sometime during November, depending on the season, the snails go into hibernation. The most populous colonies have been found in places that are relatively dry during the winter months. At three of the stations nearly all of the snails hibernated as adults. At Mutsusawa, where reproduction was delayed, about one-third of the hibernating population has been found to be immature. Before hibernation the snails tend to find places that will not be covered with water during the winter. They remain inactive until there is a suitable combination of moisture and heat. Some activity was noted in the Ido colony by the middle of February. By mid March all colonies showed some activity, proportional to the amount of moisture. When the April collections were made all the snails were active and copulating pairs were seen. In a normal year chemical control could not be administered in the area studied from 1 November to 1 April because of hibernation.

In the last Annual Report mention was made of control by winter flooding. This experiment had to be discontinued because of the threat to the wheat crop. At present this method is not practical. The changes brought about in the revamping of the drainage system at Shida practically stopped reproduction in the snail colony. A large population of adults remains, although it moved downstream a short distance.

The habitat may be suitable for egg-laying next spring unless further disturbances are initiated. If similar activities could be carried out each year a form of "natural" control might be found. In one area (Shimo-sanjo) an unplanned "lime nitrogen" treatment, used by the Japanese was given. The collection was made one week after the application of the chemical (May). Snails were very difficult to find and only 80 of those brought to the laboratory were alive. The average rate of infection at this station had been 2.5 percent during the previous four months. None of the snails in this collection were infected. In the following month, when 271 live snails were found, only immature infections were found (Fig. 2). This suggests that adverse conditions, in this case produced by a chemical, tend to kill the infected snails. Clinical data over a several year period substantiate this, in that after chemical control incidence of the disease is low until the following year, when the snail population has built up again and becomes reinfected. By migration and reproduction the snail population began returning to normal during the summer months. At present the colony is still sparse by pre-treatment standards but one more season should see it near its former level. This indicates that the chemicals should be applied once or twice a year for about two years, rather than once every 3-5 years, as has been the previous practice in Yamanashi.

It has been found that there are two periods during the year when chemicals, now available, can be used to control snails. One is after the snails come out of hibernation and before the ditches are used for irrigation. This extends from about 1 April to 15 May - 1 June. The other period is after the water is turned out of the ditches, 25 September - 1 October, until hibernation begins 1-15 November. During these periods the snails move about on a moist soil habitat and are susceptible to chemicals applied as dusts or sprays.

(2) The Chemical Control of Snails - All samples of potential molluscicides that could be obtained were tested in a cooperative project with the Japanese National Institute of Health and the Yamanashi Health Department. Laboratory screening tests were devised and the better chemicals were tried in small field plots. Three of the best chemicals from the latter tests were used in field control experiments involving about 12 miles of ditches.

The laboratory screening tests were done at the Japanese National Institute of Health by Dr. N. Ishii and Miss Y. Mitoma. A satisfactory standardized test has not yet been found. At present tests involve the exposure for 72 hours to known amounts of chemical in water. At the end of that period the snails are crushed to determine viability. In earlier experiments the exposure was for only 48 hours and viability tests consisted of washing the snails well and counting the number that showed activity when placed in water. It has been found that some substances are so irritating that the snails close up tightly and remain that way for long periods even in the absence of the chemical. Often these chemicals are not particularly lethal. An attempt has been made to put all of the screening tests on the same basis but lack of time and chemicals has made this impossible. A sample of the difference in the results of the two techniques is shown in the case of copper sulfamate. In the first set of experiments the minimum lethal concentration appeared to be 1 to 80,000. By crushing the snails it was found to be only 1 to 10,000 after even a longer exposure.

Because of the low minimal lethal concentrations certain of the chemicals were rejected for field testing. By laboratory tests the following chemicals had minimum lethal concentrations of less than 1-2,000: b-thiocyanoethyl esters (Lethane 60), Chloronitrobiphenol, "Compound 118", Copper sulphate, tribasic, DDT dust, Dichloro-diphenyl-dichlorethane (Rothane), Diethyl p-nitrophenyl thiophsophate "Hepta-Klor", Methoxychlor DDT dust, Sodium lead polyphosphate, Sodium dinitro-o-cresol (Krenite), Sodium fluoaluminate (Cryolite), Tetraethyl pyrophospnate (Nifos-T). The following had MLCs of approximately 1-2,000: Copper phosphate, DDT emulsion, Dithiocyano-diethyl ether (Lethane A:70). The following had MLCs of 1:5,000: Naphthaline, "raw", Sodium copper arsenate polyphosphate, Sodium copper polyphosphate. Hexaethyl tetraphosphate had a minimum lethal concentration of 1-6,000 but the sample was lost prior to field testing. (Note: In some instances trade names have been used here and in Table III, because the exact proportions of ingredients comprising the mixture are not known).

Twenty-five chemicals and mixtures were tested in field plots in Yamanashi Prefecture. (The chemical companies that have contributed and made this program possible include: Dow Chemical Company, E. I. duPont de Nemours Company, Hercules Powder Company, Julius Hyman & Company, Monsanto Chemical Company, Pennsylvania Salt Manufacturing Company, Rohm & Haas Company, Velsicol Corporation). Ditches about 3-4 feet wide, with many snails, were selected. Sections of the ditches 25 feet long were treated with known amounts of chemicals. Before treatment snails were collected from three 1 square foot quadrats in each plot to determine population density and the number of dead snails present. Post-treatment counts were made on similar quadrats on the 2nd, 4th and 8th days after application. The results of these tests will be found in Table III. In the case of some chemicals that showed promise different methods of application were tried, i.e., dust, spray, and mixed in the water of a dammed

Table III.

SUMMARY OF DATA ON MOLLUSCICIDES TESTED IN FIELD PLOTS

Chemical	Minimum lethal conc., 1: (1)	Rate of application and method (2)	Controls % dead	Post-treatment % dead (3)
Benzene hexachloride (isomer unknown)	Less than 2,000	100 (dust)	0.0	10.6
Benzene hexachloride (gama isomer)	5,000	10 (dust)	0.4	3.9
		25 (dust)	1.1	9.4
		50 (dust)	2.1	12.2
b-butoxy-b'-thio-cyano-diethyl ether (Lethane 384)	5,000	50 (spray)	0.6	17.8
		100 (spray)	0.6	22.0
Calcium cyanamide	Less than 2,000	500 (dust)	0.7	83.4
Chlordane	5,000	16 (spray)	1.5	13.1
		50 (spray)	11.9	19.5
		100 (spray)	19.8	27.3
		100 (In H_2O)	-	6.4
		200 (spray)	6.8	30.0
Chlorinated camphene (Toxaphene)	Less than 2,000	25 (spray)	15.5	36.4
		50 (spray)	10.3	36.9
		100 (dust)	0.0	26.2
		150 (spray)	9.4	55.6
(Penphene)	Less than 2,000	160 (dust)	1.3	29.5
Copper sulfamate	10,000	25 (spray)	0.9	2.7
		50 (spray)	0.0	8.6
		100 (spray)	0.0	7.9
Dicyclohexylamine salt of dinitro-o-cyclohexylphenol (K-604	200,000	10 (dust)	0.7	78.3
		20 (dust)	0.7	77.7
		56 (dust)	0.7	83.0
"DN-111" (20% mixture of the above	200,000	2.5 (spray)	1.3	0.9
		5 (spray)	0.0	10.4
		10 (spray)	0.0	11.1

SUMMARY OF DATA ON MOLLUSCICIDES TESTED IN FIELD PLOTS
(Continued)

Chemical	Minimum lethal conc., 1: (1)	Rate of application and method (2)	Controls % dead	Post-treatment % dead (3)
Dinitrochloro-benzene	7,000	50 (spray)	0.0	11.4
		100 (spray)	0.0	40.0
		200 (spray)	0.0	58.1
Dinitro-o-cyclohexyl-phenol	600,000	78 (dust)	0.7	93.7
		45 (spray)	1.0	91.7
"DN No. 1 (40% mixture of the above	—	45 (spray) (4)	2.2	99.0 (5)
		45 (dust)	2.1	92.7
		45 (dust) (6)	12.7	100.0 (5)
		45 (In H_2O)	3.4	96.2
		60 (dust)	1.1	87.6
Diphenylurethane	Less than 2,000	50 (spray)	0.0	1.9
		100 (spray)	0.5	1.0
"He-761"	5,000	90 (spray)	1.9	37.0
		175 (spray)	0.7	53.4
"Honspalter"	5,000	1/1500	0.7	9.4
		800 (In H_2O)	1.2	3.7
Methoxychlor DDT emulsion (Marlate) (Conc. unknown)	5,000	50 cc (spray)	19.1	29.0
		100 cc (spray)	28.1	35.0
		300 cc (spray)	24.4	45.4
Naphthaline "extract"	5,000	25 cc (spray)	4.1	15.7
		50 cc (spray)	3.7	35.6
		100 cc (spray)	0.4	16.5
o-tolylurethane	5,000	50 (spray)	0.6	1.4
		100 (spray)	0.0	6.7

SUMMARY OF DATA ON MOLLUSCICIDES TESTED IN FIELD PLOTS
(Continued)

Chemical	Minimum lethal conc., 1: (1)	Rate of application and method (2)	Controls % dead	Post-treatment % dead (3)
Pentachlorophenol (Santophen 20)	10,000	20 (dust)	0.9	18.6
		50 (dust)	1.8	32.9
		50 (spray)	0.5	60.3
		100 (dust)	0.3	35.0
		100 (spray)	0.0	66.3
		100 (In H_2O)	0.0	14.4
		200 (spray)	0.4	82.7
Pine root oil	6,000	1/1500	0.7	21.6
Pine root oil, chlorinated	6,000	200 (dust)	1.6	6.8
p-nitrophenylethyl carbonate	10,000	50 (spray)	1.9	4.4
		100 (spray)	0.0	56.3
Sodium pentachlorophenate (Sanobrite)	20,000	10 (dust)	0.0	10.2
		20 (dust)	0.0	15.1
		50 (dust)	0.0	29.6
		50 (In H_2O)	0.0	15.3
		50 (spray)	1.0	86.3
		150 (spray)	0.3	92.4
Tetraethyl pyrophosphate	3,000	50 (spray)	0.7	24.2

Total number of snails used in laboratory tests ------ 8,300

Total number of snails examined in field plot tests --72,370

(1) Laboratory test figures

(2) Grams of active ingredient per 25 ft. of ditch 3-4 ft. in width, unless otherwise indicated

(3) Average percent killed on 2nd, 4th and 8th days after treatment

(4) 375 ft. of ditch treated

(5) Snails collected 15 days after treatment

(6) 4500 ft. of ditch treated

ditch. In general, the latter method was not as satisfactory as the other two. In the case of sodium pentachlorophenate a comparable series was not tried because of the irritating nature of the dust. It appears that the insoluble dicyclohexylamine salt of dinitro-o-cyclohexylphenol is more effective than when it is mixed with a detergent (DN-111). Three of the best chemicals, sodium pentachlorophenate (as HSPA Activator), dinitro-o-cyclohexylphenol (as DN No. 1), and its dicyclohexylamine salt (as DN-111) were obtained in quantity for field control experiments. Half of each lot was used during the fall period suitable for chemical control. For comparison the remainder will be used during the spring of 1949. Extensive areas, totalling 63,600 linear feet of ditches in seven different muras, were selected for these experiments. They included ditches of all sizes, those lined with rock and/or concrete, those with banks of soil, some wet and others dry, so that a wide range of habitats were used. A description of these follows:

(a) Nirasaki - This area consisted of an "island" lying between the city of Nirasaki and the Shio River (see Fig.). Its upper boundary was formed by a large diagonal ditch from the Shio. This had a heavy and rapid flow of water and no snails were present. Four main irrigation ditches originated from this and ran more or less parallel to each other. There were also two short parallel ditches and four cross connections in the upper part of the area. The large ditch turned and also ran somewhat parallel to the irrigation ditches but turned back toward the river at the lower end. The four irrigation ditches eventually joined to drain the entire area. This formed a natural unit and if the snails were removed there would be little danger of repopulation. To get pre-treatment data on the snail population counts were made at 250 foot intervals in square foot quadrats on the parallel ditches and similar counts were made on the lesser ditches. The population was not evenly distributed, ranging from 0 to 267 per square foot. In the area 60 quadrats were counted, yielding 1732 snails, or 28.9 per square foot. Only 16, or 0.9 percent of this group were dead. Nearly all of the ditches in the area were open-box shaped, lined on the sides by perpendicular walls made of round boulders. In many places the water flow had been rapid and the snail population was low. Often a considerable population would find protection in the ample crevices made by the rocks. Vegetation had to be removed from many sections before treatment. A total of 21,850 linear feet of ditch was treated.

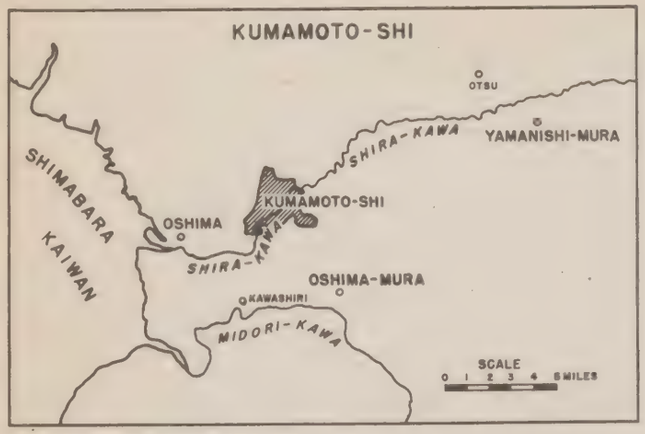
(b) Tomi-mura - At Tomi the area selected for treatment consisted of rice fields irrigated from a large reservoir. No snails were present in the reservoir and treatment started at its outlet. The system of ditches consisted essentially of the main one from the reservoir and five more or less anastomosing branches that irrigated the fields lying between two ridges. One small system of ditches from another water-shed, and well populated with snails, was found to lead into the main ditch. This area was not irrigated from the reservoir but it was treated to prevent the washing of snails into the main area. The entire area drained into a ditch that flowed through the village and it had no snails. If the area was freed of snails there should be little opportunity of repopulation. Snail counts, similar to those at Nirasaki, were made in 26 quadrats. In the total of 1071 snails collected only 6 were dead. Nearly all of this area had sloping mud banks. A great deal of vegetation had to be removed before treatment. A total of 6,425 feet of ditches was treated.

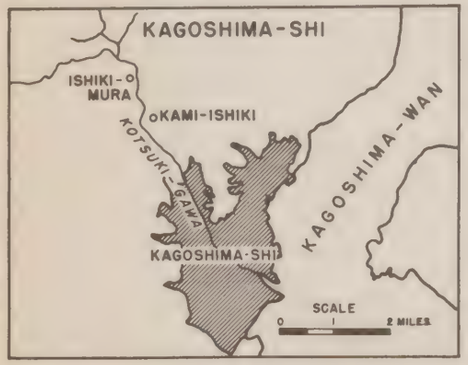
(c) Tatsuoka-mura - The area consisted of a rice-growing valley on a plateau. It had a common water source and ridges protected the sides from adjacent areas. There was a complicated system of irrigation ditches that collected into a common ditch at the lower end and fell to the floor of the main valley over a high escarpment. Snails were collected from 56 quadrats and a total of 1717 snails were found. Only three of these were dead. In most of the area the ditches had sloping mud banks but in some places rock walls were used. A great deal of vegetation had to be removed before treatment. A total of 12,840 feet of ditches was treated.

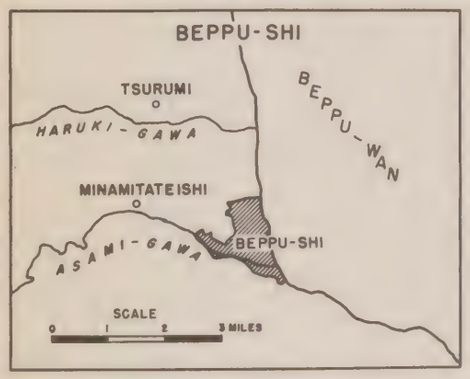
(d) Mikage-mura - This area consisted of an "island" surrounded by large permanently flowing ditches. Three main irrigation ditches ran through the area, with one cross connection. The three ditches joined at the lower end and emptied into the permanent stream. A series of lesser ditches, like ribs in a fan served the rest of the area. They had no connection with the ditches mentioned above, but emptied directly into the stream. Snail counts were made on all of these but only four were treated. The remainder will serve as a control and observations can be made on migration. Out of 19 quadrats counted there was a total of 396 snails. None of these were dead. Vegetation was plentiful and had to be removed before treatment. The banks were muddy slopes. A total of 3,450 feet was treated.

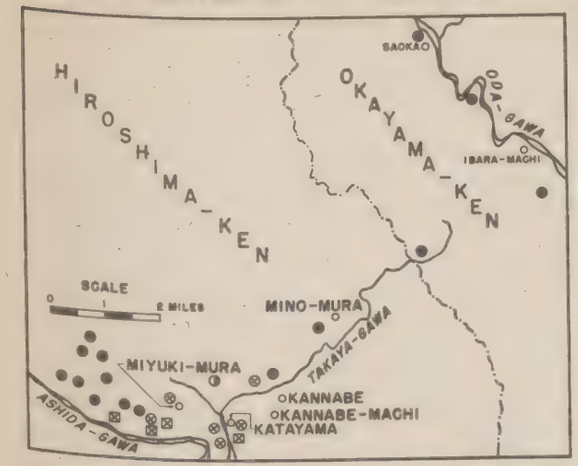
(e) Tanooka-mura - The area consisted of two drainage systems, one above a permanent stream and the other below it. The system above drained into the stream and could have been a source for the repopulation of the lower area. The lower group of ditches consisted of six fan-like ditches that joined at the lower end to drain the area. The upper system was made up of two long ditches with short branches. If this area was cleared of snails there seemed to be little chance for repopulation from adjacent fields. From 36 quadrats 1708 snails were recovered and of these 14 were dead. Vegetation was plentiful and had to be removed. For the most part the banks were composed of dirt but one section consisted of a long, narrow concrete conduit. A total of 10,245 feet was treated.

PARASITOLOGICAL STUDIES - 1948









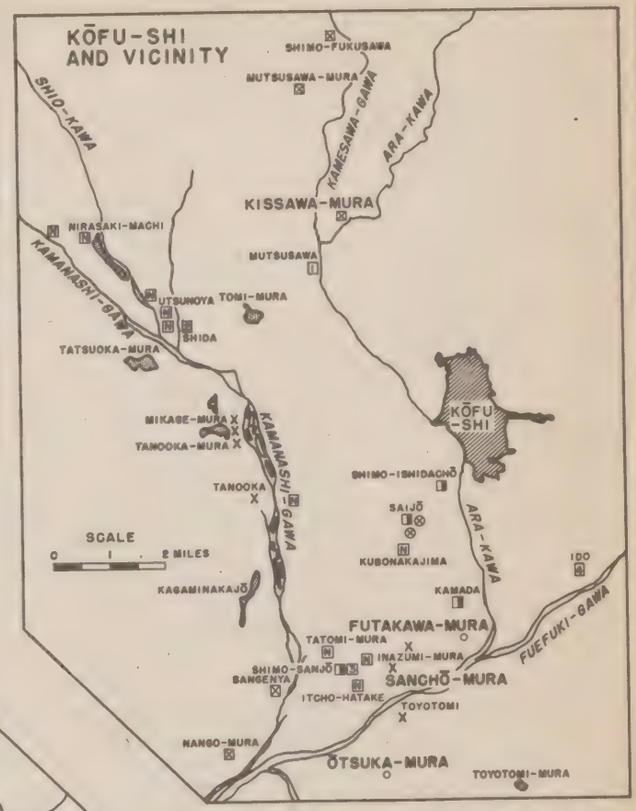


FIG. 1

(f) Kagami-nakajo - The area treated consisted of one large ditch. At its upper end it came from a large permanent stream. There were a few snails on the latter so it may be possible to determine how soon the treated area becomes repopulated from the untreated area above. The ditch begins as a concrete sluice, about four feet wide with perpendicular walls about two feet high. Further down the walls were made of stone and concrete. The ditch had been in use long enough so that a heavy growth of algae was present. At the village the ditch was covered and contained no snails. Beyond the village the walls were usually made of round stones. The latter offered better protection and contained most of the snails. From 23 quadrats only 112 snails were found but none of them were dead. Vegetation had to be removed in only a few places. A total of 4,900 feet was treated.

(g) Toyotomi-mura - This area consisted of a small valley relatively high on a hillside. Just above and to the side of the rice-growing area in this valley there were four small ponds or reservoirs. The lower pond contained Oncomelania and offered a new type of habitat for treatment. The most important part of the area consisted of the main ditch and the small tributaries that drained the fields. If the area was cleared of snails there appeared to be little opportunity for repopulation. From 11 quadrats only 47 snails were found but none were dead. Most of the ditches had mud banks but some had rock walls. Vegetation was plentiful and had to be removed. A total of 1890 feet was treated.

DN-111, a 20 percent mixture of the dicyclohexylamine salt of dinitro-o-cyclohexylphenol, was applied at the rate of 5/8 pound per 100 linear feet. DN No. 1, a 40 percent mixture of dinitro-o-cyclohexylphenol, was applied at the rate of ¼ pound per 100 linear feet. HSPA Activator, 79 percent sodium pentachlorophenate, was applied at the rate of 260 grams per 100 linear feet. Seventeen to 26 days after treatment counts from the same number of quadrats were made to determine the percent killed (see Table IV). DN-No. 1 appeared to be the most effective. Plants were not injured by the chemicals but fish, annelids, arthropods, etc. were killed. Linear measurement has proved an over-simplification and in the future the area involved will have to be considered. Populations in loose stone walls were the most difficult to irradicate. Nearly all of the live snails recovered were brought to the laboratory, where they soon died. This may indicate that many of these snails will not be able to survive the winter. If complete control is to be attempted adequate maps and surveys will be the first prerequisites. The personnel needed for a snail control team has been determined.

Table IV. Summary of Data on Snail Control Under Field Conditions

Area	Ditch Size	Type	Chemical	Percent Killed
Toyotomi	Small	Dirt	DN-111 dust	48.8
Tanooka	Small to medium	Dirt	DN-111 spray	33.9
Tomi	Small to medium	Dirt	DN-No. 1 spray	73.4
Tatsuoka	Small to medium	Dirt	DN-No. 1 spray	75.6
Kagami-nakajo	Large	Concrete & stone	DN-No. 1 spray	60.3
Mikage	Medium	Dirt	HSPA Activator	74.7
Nirasaki	Large	Stone	Activator	37.8
Controls	Small to large	Dirt and stone	None	1.1

Total footage treated	63,600
Total snails before treatment, treated area	6,725
Total snails from same number of quadrats, post-treatment	5,110
Percent less snails recovered after treatment	24.0
Percent dead snails in pre-treatment counts	0.6

(3) Seasonal Cycle of S. japonicum infections in the Intermediate Host - Each month more than 2,000 snails from four collecting stations in Yamanashi have been crushed and examined for trematode infections. During the year 823 S. japonicum, one ophthalmo-xiphidiocercaria and one monostome infections were found in 20,034 snails. The data in Table V and Figure 2 indicate that a few infections may be picked up at any time that the snails are active. During this period there were two periods when most of the snails became infected. The major one was in June and July and there was a smaller one in October and November. During the winter months the development of the sporocysts remains stationery. (Essentially similar results were also obtained in laboratory infected snails in 1947-1948 except that the sporocysts continued to develop slowly during the winter months. This difference is to be expected in view of the different ecological conditions to which the snails were exposed.) During the winter of 1947-1948 the infections were predominantly immature or just reaching cercarial production. The summer of 1948 was cooler and there was more rain than in 1947. This may account for the carry-over of a rather large number of mature infections into the winter months of 1948. The difference in the infections, in

MONTHLY EXAMINATION OF SNAILS FROM YAMANASHI-KEN

PERCENT INFECTION AND APPROXIMATE AGE

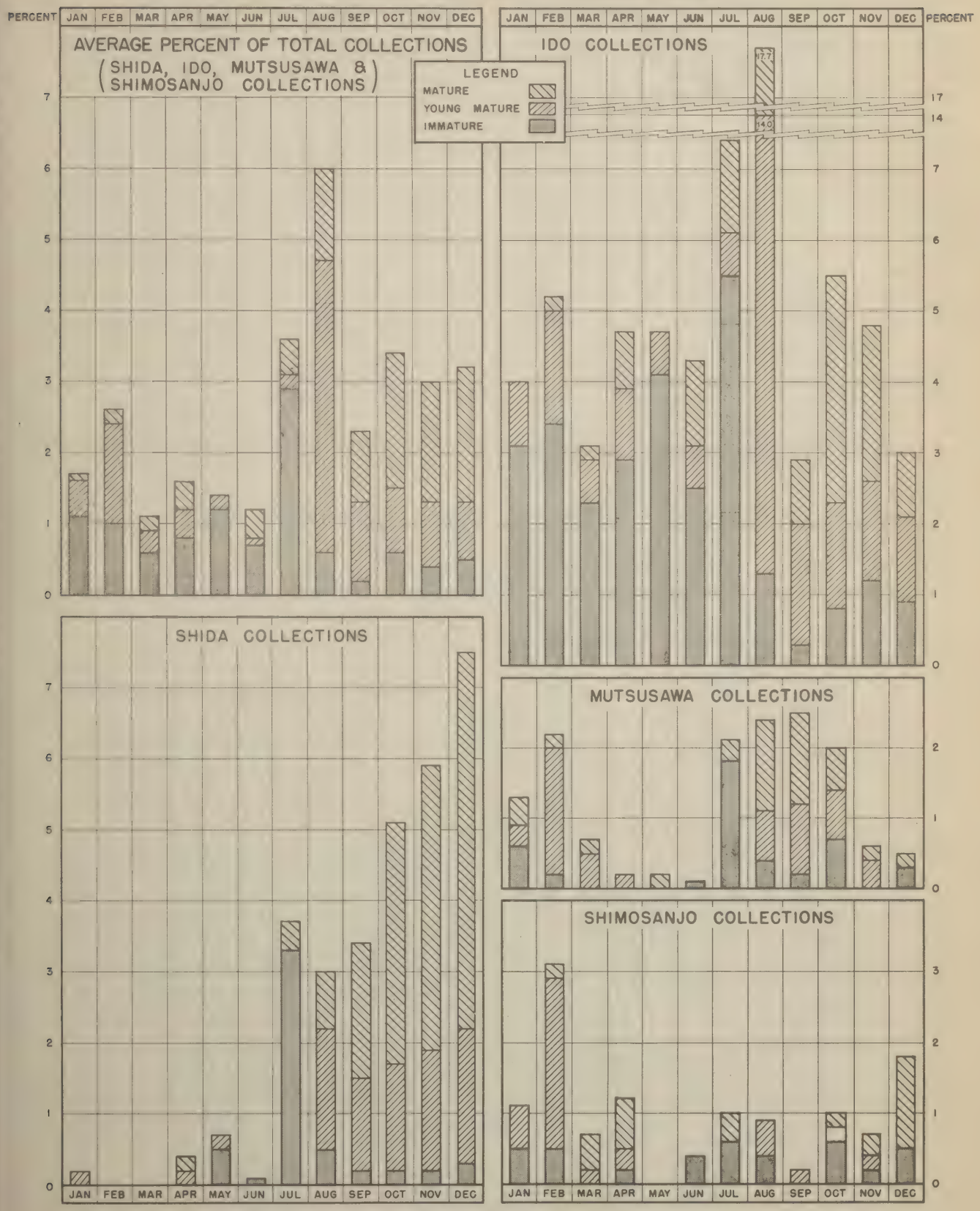


FIG. 2

the two seasons, is clearly shown in Figure 2. If these mature infections survive the winter, next spring will probably see an increase in the rate in man. In the winter of 1947-1948 relatively few snails were capable of producing cercariae (see Table V and Figure 2) and the average age was low (see Table VI). In April and June about one-third of the infections were producing cercariae. In 1948 there were large numbers of snails with cercariae during the early winter months. During the period from October to March there would be little opportunity for cercarial production because the snails are hibernating in relatively dry places.

Table V. Summary of S. Japonicum Infections By Age Groups (1948)

Month	Number of infections by age group[x]									
	a	b	c	d	e	f	g	h	i	Totals
January	0	4	8	4	3	4	11	2	0	36
February	0	1	7	4	7	4	32	3	0	58
March	0	5	1	3	6	0	8	5	0	28
April	0	5	5	5	1	2	10	10	0	38
May	0	3	0	8	21	13	7	1	0	53
June	0	4	6	8	3	0	4	9	0	34
July	0	4	50	12	0	1	4	13	0	84
August	0	0	3	9	2	6	149	48	1	218
September	0	0	0	1	2	1	25	23	1	53
October	0	1	2	2	6	3	22	43	1	80
November	0	3	3	1	0	1	20	38	0	66
December	0	2	5	3	1	1	19	43	1	75

[x]
a - 2 wks
b - 3 wks
c - 4-5 wks
d - 6 wks
e - 7 wks
f - 8 wks
g - 10 wks
h - 20 wks
i - 40 wks

These ages are based on the appearance of the infection and the time required for development to reach the stage present under optimum conditions. Immature includes up to 8 weeks, young mature up to 10 weeks and mature infections include all others. (Adapted from Faust and Meleney (1).

Some authors have stated that the snails live five or more years and if infected when young they retain their infection throughout their life span. The data presented in Figure 2 suggest that the schistosome infection is relatively short lived. In the winter of 1947-1948 the infections were found to be predominately young. The snails with mature infections of the previous summer had disappeared. The young infections of the winter died out during the spring and early summer. In the fall of 1948 a large number of mature infections remained, except at the Ido station. At Ido most of the infections acquired in June-July had disappeared by September, following the pattern of all of the stations in the previous year. It would appear that the infections acquired late in the season may live for about 10 months. Usually the infections acquired in the spring and early summer live only 3-4 months. If infection was prevented for one season the carry-over into the next summer would apparently approach a vanishing point.

Most of the night soil is applied in November (wheat planting), January-February (wheat) and in July-August (rice). It will be noted in Figure 2 there is no obvious relationship between these and the rate of appearance of young infections. In October and early November the fields are prepared for planting wheat. In June they are plowed again in preparation for rice planting. Most of this is done with cattle that are heavily infected with S. japonicum. The small number of infections that appear

Table VI. Summary of Data Obtained in the Monthly Examination of Snails from Yamanashi (1948)

Month	Number of snails	Number of infections	Average % infected	Average age of infection
January	2230	36	1.7	7.8
February	2251	58	2.6	9.0
March	2341	28	1.1	9.3
April	2295	38	1.6	10.3
May	2317	53	1.4	7.5
June	2430	34	1.2	9.7
July	2260	84	3.6	7.6
August	2701	218	6.0	12.0
September	2323	53	2.3	14.7
October	2464	80	3.4	15.1
November	2204	66	3.0	15.1
December	2218	75	3.2	15.4
Totals	28034	823		

Note: Since number of snails was not exactly the same from the four collecting stations "average percent" is given above, rather than percent based on total number of snails.

Average age is given in weeks.

in the fall may be acquired during the fall plowing. A much greater number appear shortly after the spring plowing. The relative amount of moisture present during the two periods may account for the difference in the number of new infections. Clinical evidence indicates that most of the "kabure", the reaction said to be associated with the passage of cercariae through the skin, is acquired during the time the fields are prepared for rice, its planting and early cultivation. It is possible that part or most of this may be caused by a bird schistosome. The August peak of cercarial production seems to be of less importance in the farmer's routine. The greatest number of clinical cases is reported in September. Military maneuvers during this time would be dangerous.

(4) Laboratory Infections of the Snail, Oncomelania nosophora - While considerable information on the age, growth of the infection and shedding of cercariae of S. japonicum can be gleaned from studies of the snail host in the field it is desirable that such information be supplemented by data from laboratory experimentally infected snails. Attempts were made to secure laboratory infections of snails with miracidia from human or dog stools. Snails were collected from lightly infected areas and maintained in the laboratory long enough to eliminate the possibility of a previous infection. It was planned to expose laboratory snails to varying numbers of miracidia in an attempt (1) to determine the number of sporocysts and cercariae produced by a single miracidium; (2) ascertain the effect multiple infections had on the numbers of cercariae produced; (3) secure information on the shedding pattern for cercariae to permit comparisons with similar infections in O. quadrasi from the Philippines and Australorbis glabratus, the intermediate host for S. mansoni; (4) compare the numbers of cercariae obtained after resting between periods of submersion with those secured by constant submersion once the snail began to shed cercariae, including total numbers shed and length of time over which shedding takes place. Such information on the length of the shedding period should be of value in determining adequate control measures necessary for snails and/or cercariae during possible military operations.

The results obtained thus far are disappointing largely because of the high mortality rate among the snails so that adequate numbers for comparison were not obtained. In general, it appears that a greater proportion of snails became infected if they were exposed to more than one miracidium. There is

experimental evidence to show that more than one miracidia is capable of penetrating the snail. As many as three well developed mother sporocysts have been recovered from a snail originally exposed to five miracidia. These mother sporocysts were equal in size and development. They have been found along the regions of the upper, middle and lower intestine and one was found at the point of juncture of the intestine and the liver. All daughter sporocysts recovered in these studies were found in the liver. From observation it appears that the daughters migrate to the proximal portion of the liver and from there on toward the distal portion depending on the number of daughter sporocysts present. Development of both the mother and the daughter sporocysts was normal in these experiments (according to the age classification used in Table V) until the infection was about five weeks old. (At this time most of the sporocysts found had developed to a stage where small cercarial masses were beginning to form within the daughter sporocysts). After this time development appeared to be retarded and the infection was over 12 weeks old before the first cercariae were shed.

Numbers shed by a single snail range from 1 to 273 on different night. In similar experiments carried out last year as many as 1442 cercariae were shed from a snail in one night. These infections had been carried over through the winter.

Total numbers of cercariae shed do not appear to be connected with the original number of miracidia penetrating the snail. The largest total number of cercariae shed (626) came from a snail originally exposed to one miracidium, but 596 were recovered from a snail exposed to three miracidia and counts of over 300 and under ten have been made on snails exposed to one, two and three miracidia. Considerable variation occurred in the numbers of cercariae produced daily without or after rest. The shed was greater when the snails were continuously submerged.

Some idea of the numbers of cercariae produced from a single miracidium may be gleaned from one snail which produced 626 cercariae before death. All of the 41 sporocysts found were examined microscopically and were found to contain mature cercariae as well as immature cercariae at the six and seven week stages. Many of the sporocysts had obviously contained mostly mature cercariae, but were so fragile that the pressure exerted when the snail was crushed had released many (1400-1500) free cercariae by rupture; some were seen in the process of leaving the ruptured sporocysts. Examination of the whole sporocysts showed that the numbers of mature cercariae and developing cercariae within varied from sporocyst to sporocyst but all contained many more than the 20 arrived at in former examinations. Two sporocysts were removed and the mature and immature cercariae in each one counted. There were no signs of development under the six week stage - no five week germ balls nor single germ cells were observed among the remains of the dissected snail. Of these two sporocysts one was picked from among those which appeared to have the maximum number of developing and mature cercariae within and the other one from among those with the minimum number. If these two totals are averaged (148) and multiplied by the number of remaining sporocysts (41) a total of 6068 potential cercariae is reached. Added to the 626 already shed a total of near 7,000 is obtained from the infection of one miracidium. These estimates furnish the most accurate count to date of numbers produced by a single miracidium.

(5) Examination of, and Infection Experiments on, Other Potential Snail Hosts of S. japonicum - Several species of snails exist in Japan, which, according to Japanese malacologists, are closely related to O. nosophora, the only known intermediate host of S. japonicum in Japan. Five related species, Blanfordia fukuiensis, B. castanea, B. japonica echinizensis, Paludestrina yoshimurai and Schistosomophora minima were collected in Fukui and Ichikawa Prefectures in 1947 and again in 1948. (See Fig. 3). The natural habitats of the several species were carefully noted. These snail collections were used both for experimental exposure to miracidia of S. japonicum as a means of determining their potentiality as intermediate hosts of this parasite, and for morphological and taxonomic studies on the snails. Both B. fukuiensis and B. castanea occur on moist, slime-covered cliffs kept wet by a constant dripping of water. Both P. yoshimurai and S. minima are found in small flowing streams which sometimes flow into paddies. B. japonica echinizensis was limited to damp areas which lacked any evidence of water accumulations. It was concluded that because of their ecology they could not serve as the intermediate host of S. japonicum. A full report on the morphology, taxonomy and biology of these species is being prepared in collaboration with R. T. Abbott of the U. S. National Museum.

None of the four species considered experimentally were found to be naturally infected with S. japonicum, and neither did they acquire infections when snails were exposed individually to miracidia. There was evidence that penetration did occur, as a few degenerating mother sporocysts were found. Comparable attempts to infect O. nosophora were successful and serve as controls on the procedures used. Both juvenile and adult snails were used. The conclusion is that the snail species under consideration do not represent potential intermediate hosts of S. japonicum. Experimental data are included in Table VII.

Table VII. Summary of Crushing and Experimental Infections of Potential Snail Hosts for S. Japonicum

Species	No. Examined by Crushing 1947	1948	Total	No. Exposed 1947	1948	Total	No. Positive 1947	1948	No. of days following Exposure That Snails Were Examined
Blanfordia fukuiensis	117	68	185	12	199	211	0	0	10 - 82 days
B. castanea	75	37	112	12	63	75	0	0	13 - 85 days
B. japonica echinizensis	369	-	369	80	-	80	0	-	15 - 82 days
Paludestrina yoshimurai	70	73	143	27	73	100	4[x]	0	8 - 71 days
Schistosomophora minima	179	90	269	87	303	390	8[x]	0	7 - 50 days

[x] Degenerating Mother Sporocysts Only.

It should be pointed out again that there is no evidence of the existence of schistosomiasis in the Fukui area. It is believed that the report of positive cases contained in TB MED 160 (2) is in error and was undoubtedly due to a faulty translation.

(6) Protection Experiments with Copper Oleate Ointment Against Schistosomiasis - During 1948 the effectiveness of copper oleate was tested as a protective agent against the penetration of cercariae of S. japonicum. While this ointment was designed to protect man against schistosomiasis, of necessity mouse protection tests were the basis for this study. The copper oleate ointment was provided by Colonel William D. Fleming through the courtesy of Colonel Rufus L. Holt of the AMDR&GS. The problems included the question of how successfully it could be used as a skin ointment and the mode of anti-cercarial action both in vitro and in vivo.

Copper oleate is a semi-solid, dark blue, sticky substance which is insoluble in water but freely soluble in ether and other fat solvents. At body temperature it is a sticky, highly viscid semi-liquid. It is most easily applied to the skin in the ether solution. The ether evaporates in five or ten minutes leaving a thin, sticky film which is difficult or impossible to wipe off completely. Soap and water will not remove it and, in fact, the only satisfactory method of removal is by swabbing with ether. This obviously would be a drawback in use with troops.

Schistosome cercariae tested in vitro lose their motility after 15 to 30 minutes contact with a copper oleate film. Soluble materials seem to play no part in this effect inasmuch as water "saturated" with copper oleate has no untoward effect on the cercariae. Besides the "chemical" action cited above, it was presumed that the oleate might also act as a mechanical barrier. Mice were used for in vivo testing of the material. The back of the animal was completely shaved and covered with a film of copper oleate. The prepared surface was then superimposed over a water bath consisting of a paraffin-lined watch glass set into a wooden block. One set of control animals was shaved and exposed without the protection of the oleate film. Another control involved use of oleate film without including cercariae in the bath; this procedure was used to detect any deleterious effects on the mice resulting from the chemical and procedures employed. Application and removal of the oleate was timed, so that ether as a solvent would not have a chance to effect the cercariae.

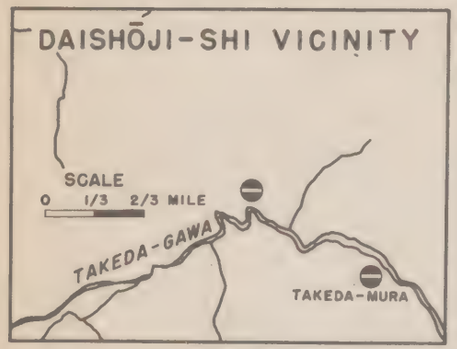
The outcome of the experiments appear in Table VIII. The copper oleate ointment apparently gives good protection, since 34 out of the 36 mice exposed were uninfected at autopsy. The two positive mice yielded only seven (2 and 5 respectively) S. japonicum in contrast to the unprotected controls where up to 99 parasites were recovered from a single animal. Apparently the protection is not due to a mechanical effect alone as other control mice protected by a paraffin-vaseline or pure vaseline mixture became infected (see Table VIII). Toxicity studies which were carried on to determine possible effects of copper oleate on the survival of mice gave no indication of any marked deleterious effect. It would appear, therefore, that copper oleate has considerable value in protecting against the penetration of S. japonicum cercariae in mice. Further experiments should be carried on in man with this copper oleate ointment using the bird schistosomes that cause "swimmers itch".

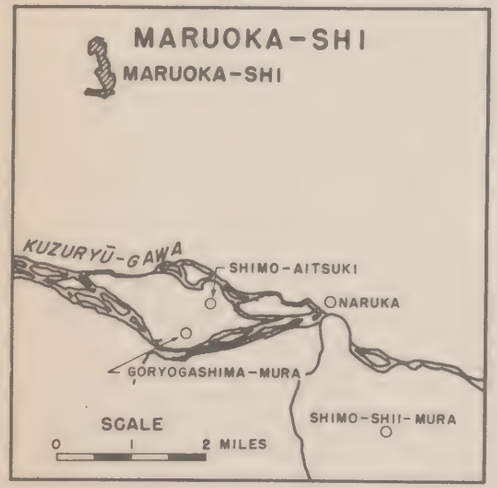
(7) The Occurrence of Schistosome Dermatitis in Shimane Prefecture - A severe type of dermatitis, "koganbyo" (lake-side disease) was found in Shimane Prefecture in a rice growing district west of Shinju Lake. Each year 1000-2000 cases are reported. The people are severely handicapped and often

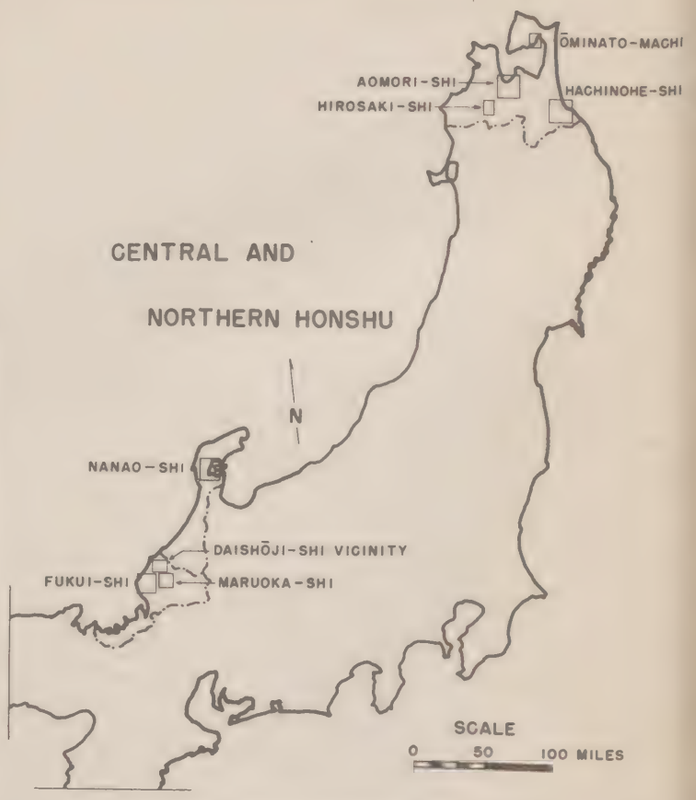
INTESTINAL PARASITISM

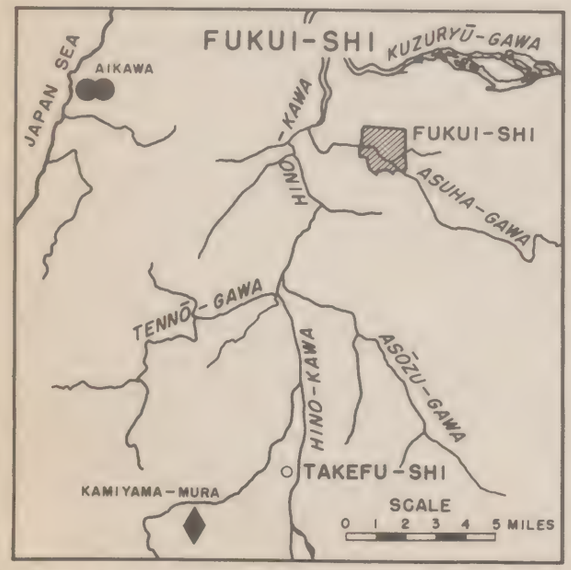
FUKUI PREFECTURE AND VICINITY

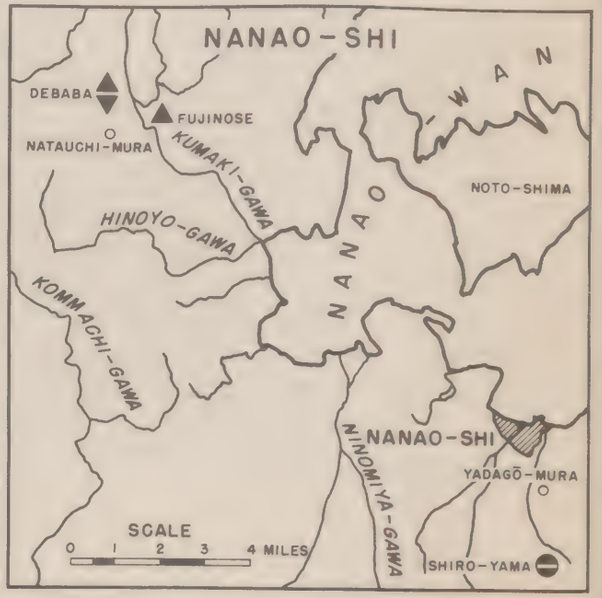
AND AOMORI PREFECTURE – 1947-48

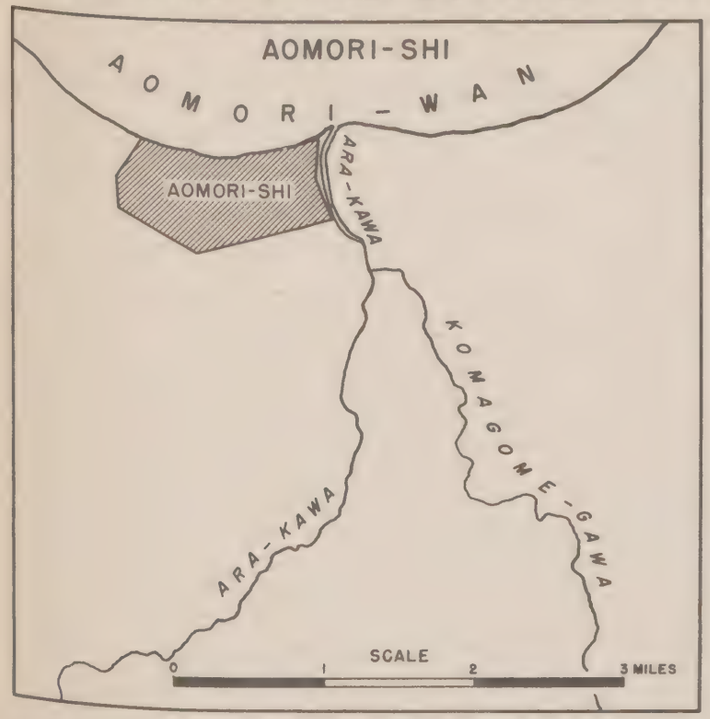


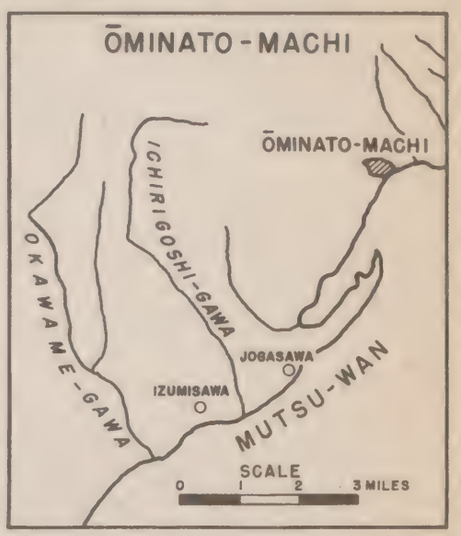


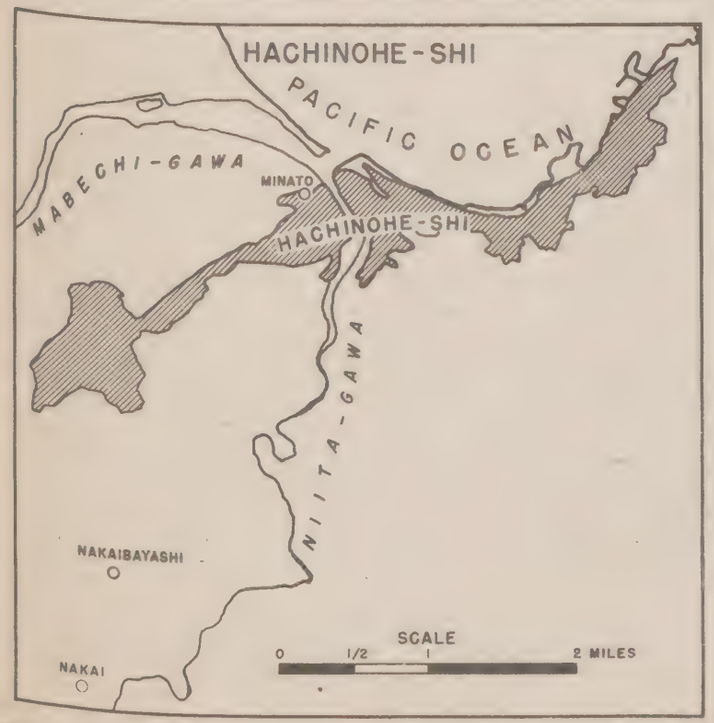












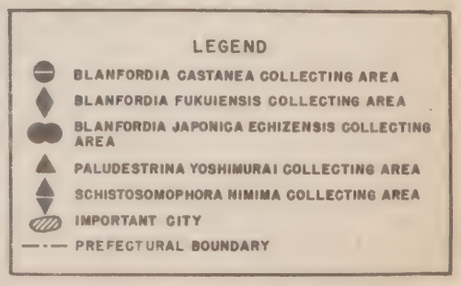


FIG.3

incapacitated by the infections. Examination of the eggs and evidence obtained by interested Japanese indicates that the dermatitis is caused by the cercariae of a species of Gigantobilharzia, a schistosome of birds. At present it appears that the epidemiology is somewhat as follows: (1) The snail host, Segmentina nitidella, lays its eggs in April-June and the adults die by the last of July or mid-August; (2) The new generation of snails is not infected until migrating birds come into the area from October to March; (3) A new generation of snails is born after the birds leave; (4) The schistosome infections in the old snails mature by rice planting and cultivating season. This cercaria may account for the skin manifestation ("kabure") often said to be associated with S. japonicum infections.

Table VIII. Summary of Protection Afforded by Copper Oleate Ointment Against Penetration of Cercariae of Schistosoma Japonicum

	No. Exposed	Positive No.	Positive %	Negative No.	Negative %
Experimental Mice:					
Protected by Copper Oleate	36	2	5.6	34	94.4
Control Mice:					
Exposed to cercariae	44	35	79.5	9	20.5[x]
Protected by paraffin-vaseline mixture	3	3	100.0	-	-
Protected by vaseline	3	3	100.0	-	-

[x] Mice died so soon after exposure that it is probable that any Schistosoma japonicum present were missed.

(8) Immunological Studies on S. japonicum - During the calendar year 1948 plans were made for the study of skin tests on S. japonicum cases and the evaluation of this antigen. Other immunological problems included plans for cross immunity studies on schistosomiasis.

A cooperative program was initiated with Dr. Sugiura of Kofu to evaluate a skin test for the early detection of schistosomiasis japonica. In order to evaluate the potentialities of the antigen it was decided to run a preliminary series of tests on the following groups: schistosome cases passing ova, persons negative for schistosomiasis but positive for other helminths, individuals negative for helminths, those positive for other trematodes (such as Clonorchis) and persons with various allergies. It was planned to test the antigen in the following dilutions: 1:1000, 1:5000, 1:10,000, 1:20,000 and 1:40,000. Controls consisting of sterile saline and merthiolate (1:7500) were included. Both saline and alcoholic fractions were tested. In these tests approximately 0.01 cc was inoculated intracutaneously on the flexor surface of the forearm. An increase of 3 mm in the diameter of the wheal within 10 minutes as compared with the control was interpreted as a positive test. In other cases the wheal was measured just after the injection and at the end of 10 minutes. While there are certain objections which can be raised concerning these methods both have received wide acceptance. (3).

The antigen was prepared by infecting rabbits and mice. After 6-8 weeks the animals were sacrificed and the adults harvested using a modification of the technique described by Yolles et al (4). The worms were washed in sterile saline, then in sterile distilled water in order to remove the blood from the gut of the parasite. They were then dried in vacuo and sealed. The antigen was weighed out and extracted by saline or alcohol to give a dilution of 1:100. The test dilutions were made from this. Merthiolate was added in dulution of 1:7500 as a preservative. Controls consisted of merthiolate in saline.

The results obtained can only be regarded as preliminary. The standard for a positive test was an increase of 3 mm in the wheal in 10 minutes - as compared with the control. While five dilutions were used the results presented here were based upon only 1:5000, 1:10,000 and 1:20,000. It appears likely that a dilution of 1:10,000 will prove to be the most efficacious concentration. Twenty-one or 88 percent of the 24 persons who were passing eggs of S. japonicum, were positive when tested with the saline extract. It should be borne in mind that many of the positive cases are so light that the eggs are recovered only after repeated examinations. Twenty-four of 30 non-schistosomiasis cases were negative with saline while another three were doubtful, making a total of 27 out of 30 who gave "negative" reactions with the saline antigen. Over 87 percent of 54 controls from a non-schistosomiasis area were also negative when tested with the saline fraction. In no instance did the alcoholic fraction yield as good results.

It is hoped that a continuation of this problem will result in the production of an antigen which will prove to be useful in detecting the early stages of schistosomiasis japonica and perhaps some of the more refractory cases as well. Attempts will be made to prepare an antigen from cercariae.

Epidemiological Studies in the Far East -

(1) A Survey for Intestinal Parasites in Occupation Personnel - An attempt has been made to determine the extent to which Occupation personnel have contracted parasites during their tour of duty in Korea and Japan. People were divided into two categories - those who were new arrivals, i.e., had been overseas less than three months, and in contrast, all of those who had been overseas longer than three months. The infection rates were then compared.

During 1948 it was possible to examine 327 Americans in Korea. Eighty-six of these had been overseas less than three months while the remainder exceeded that interval. A similar grouping of Occupation Personnel in Japan from 1946 to 1948 shows 156 fell into the first category while 1343 had been here in excess of three months. Only the more common parasites are listed in Table IX.

Table IX. Comparison of the Incidence of Intestinal Parasites In Occupation Personnel in Japan and Korea

	Americans in Japan (Total)				Americans in Korea			
	1 to 3 months		Over 3 months		1 to 3 months		Over 3 months	
	No.	%	No.	%	No.	%	No.	%
Number examined	156		1343		86		241	
Number parasitized	50	32.0	484	35.9	19	22.1	73	30.3
Number with helminths	8	5.1	68	5.1	1	1.2	20	8.3
Number with protozoa	44	28.2	446	33.2	18	20.9	62	25.7
Helminths:								
Ascaris	1	0.64	16	1.2	0	0.0	5	2.1
Whipworm	4	2.6	23	1.7	0	0.0	3	1.2
Hookworm	4	2.6	27	2.0	1	1.2	11	4.6
Strongyloides stercoralis	0	0.0	0	0.0	0	0.0	1	0.4
Hymenolepis nana	0	0.0	1	0.07	0	0.0	0	0.0
Metagonimus sp.	0	0.0	0	0.0	0	0.0	1	0.4
Protozoa:								
Endamoeba histolytica	8	5.1	73	5.4	3	3.5	12	5.0
E. coli	26	16.7	220	16.4	11	12.8	34	14.2
Endolimax nana	18	11.5	214	15.9	6	7.0	25	10.4
Iodamoeba butschlii	0	0.0	2	0.15	0	0.0	1	0.4
Giardia lamblia	7	4.5	78	5.8	3	3.5	9	3.7
Chilomastix mesnili	0	0.0	0	0.0	1	1.2	0	0.0

It must be recognized that the figures available are limited and that the overall picture may change as more data become available. At present the figures show that among those individuals who have been overseas more than three months, there has been a slight increase in the number parasitized. In Japan there was an apparent increase of only about 4 per cent while in Korea the difference is more than double that figure (see Table IX.

Among those in Japan the increase was primarily associated with the protozoa, especially Giardia lamblia and Endolimax nana. There was no significant change in the incidence of amebiasis. On the other hand, American personnel in Korea showed a greater increase in parasitic worms, especially ascaris, whipworm and hookworm (see Table IX). The protozoa also showed a slight general increase including Endamoeba histolytica (3.5 to 5 per cent). It appears that there have been at the most only slight increases in the incidence of intestinal parasites among the Occupation Forces in Japan and Korea.

(2) Surveys for Intestinal and Blood Parasites in the Japanese - A series of extensive surveys for intestinal parasitism in Japan has been carried out during the past 18 months as a cooperative project with the Japanese National Institute of Health. Although the work accomplished in 1947 is not included in the report the areas surveyed will be mentioned. Population samplings have been made in widely diverse geographical areas including Fukui, Aomori, Yamanashi, Okayama Prefectures, the island of Kyushu (Fukuoka, Saga, Kumamoto, Kagoshima and Oita Prefectures) and the Tone Valley. Maps of these areas showing the hamlets, villages and cities are appended. In cases where surveys for snails have been made the regions examined have also been shown. (Figures 1, 3) It is contemplated to conduct surveys on Hokkaido, Shikoku and the fifth known area for schistosomiasis near Numazu. With these, and perhaps one other, it is hoped to round out the parasitological picture for Japan.

In a series of epidemiological surveys such as has been undertaken there are a number of correlations upon which it is hoped to obtain information. Seldom has it ever been possible to undertake such extensive and comprehensive surveys elsewhere in the world. The stage is set here so that a high degree of cooperation can be obtained.

Methods - To accomplish the desired objectives an epidemiologic data sheet is completed on each person examined, the questioning being done by Japanese physicians. This sheet is reproduced as Figure 3. The same Japanese physicians perform a brief physical examination on each person, which includes checking for evidence of hepatomegaly, splenomegaly, anemia, jaundice, ascites and lymphadenopathy. The general state of health is recorded. Finally, in some instances, blood smears for malaria or filariasis, as well as perianal swabs for pinworm, are made.

A minimum of 1200 persons and a maximum running to over 3000 have been examined in each geographical area where a survey has been carried out. Every effort is made to secure representative samplings of both sexes as well as all age groups and economic strata. In many instances it has been necessary to permit occupation of the group to be a single limiting factor.

Following the physical examination, the stool specimen is examined by the MGL (4) and AMS III (5) techniques for worms and protozoa. (Both of these techniques are modifications of the original Telemann acid-ether centrifugation method). Finally, direct smears are examined to obtain figures that have some comparative value with earlier surveys, and an arbitrary rough calculation of the density of parasitism made. Throughout the surveys an attempt to secure a constant line has been (and is being) made by using the same techniques, the same supervisors, the same technicians, and the same Japanese physicians.

A parasite density index or factor has been used in an attempt to secure information on the degree or intensity of the infection, something which is not usually tried on more than one or two species of parasites (6). While the index is an arbitrary figure, nevertheless the results can be compared and Figures 4 to 10 show clearly how the picture varies from one region to another. The index is reached as follows: the number of eggs (or cysts) in each microscopic preparation is recorded

Symbol	Actual Count	Index Figure
/	1 - 9	5
//	10 - 49	30
2/	50 - 99	75
3/	100 - 199	150
4/	200 - 399	300
5/	400 /	500

The total number of cases in each category is multiplied by the index figure. These figures are totalled and divided by the number of infected persons. The resulting figure is the parasite density index or factor. In this way a rough but usuable index is obtained.

Information sought - Does the presence of one parasite predispose to infection with any other parasites, or render such infections more persistant? What is the correlation, if any, between incidence and density of parasitism? What evidence is there of immunity? Does it develop with age or is it non-existant? How much do the parasites predispose to other diseases, such as tuberculosis? What is the relationship between the methods of dispersal of a given species of parasite and the terrain, the use of nightsoil or even the incidence of parasitism? In one community in Ibaraki Prefecture hookworm was the cominant parasite while in a continguous hamlet Trichostrongylus dominated and hookworm was the fourth most prevalent worm. How can such differences be explained? What are the various factors influencing the comparatively low rate of incidence of Endamoeba histolytica? The overall rate obtained in Japan thus far in the surveys does not seem to be out of line with that reported for the United States.

Is there any significant relationship between climate and parasitism? Japan extends from approximately 31° to 45° N. Latitude (from Jacksonville, Florida to Montreal, Canada is a comparable distance) and racial and occupational differences between the two extremes are less perhaps than in any similar geographic range. Is there a northern boundary of hookworm infection? Ominato, at slightly above the 41st parallel yielded an incidence of 11 percent infections with this worm.

What is the potential danger of introducing unfamiliar parasites into other areas of the world? In northern Honshu there are areas in which 90 percent of the population examined yielded eggs of Trichostrongylus, a phasmid nematode not known to occur in the Western Hemisphere.

EPIDEMIOLOGICAL SURVEY

No.....

PERSONAL DATA — For IBM Coding Only

Specimen No.....Name..... 1-5

Prefecture.....City.....Section..... 6-9

Age.....Sex 1. Male.....2. Female..... 10-11

Occupation: 1. Farming....Dry....% Paddy....% 2.Fishing..... 12

3. Merchant....4. Clerk....5. Other....6. Laborer.... 13

7. Professional....

KIND OF WATER USED FOR DOMESTIC PURPOSES

1.Tap 2.Well 3.River 4.Stream 5.Ditch 6.Pond

Drinking 14

Washing 15

Bathing 16

PREVICUS RESIDENCES

11. Honshu..... 12.Kyushu....13.Hokkaido.....14.Shikoku.... 17-18

21. Korea....22.New Guinea.... 23.Okinawa....24. Formosa....

25.Philippines....26. China....27.Others....

28.Manchuria....

DIAGNOSIS

....10.Ascaris 20.Tt. 19-22

....30.Hw. 40.Tricho 23-26

....50.Schisto 60.E.histo 27-30

....1.E.coli ...2.E.nana....3.Giardia ...4.Chilo ...5.Ioda.

....1.Clon ...2.Trema egg ...3.Meta ...4.H.nana ...5.Strong. 31-35

36-40

PERIANAL SWAB Pinworm: 1. Present.... 2. Absent.... 41

TYPE OF STOOL

10. Normal..... 20.Diarrheal..... 30.Constipated..... 42-43

21.Soft.....

22.Watery.....

23.Mucus.....

24.Bloody.....

HISTORY (Past six months)

	1. None	2. Seldom	3. Often	4. Chronic	
Nausea or vomiting	....	.....	.....	.....	44
Diarrhea	....	.....	.....	.....	45
Abdominal pain	....	.....	.....	.....	46
Perianal itching	....	.....	.....	.....	47
Constipated	....	.....	.....	.....	48
Malaise	....	.....	.....	.....	49
Chills & Fever	....	.....	.....	.....	50
Others	....	.....	.....	.....	51

RESPIRATORY DISORDERS — Col.

1. Yes 2. No 1.Yes 2.No 1.Yes 2.No

Cough .... Bloody sputum ..... Chest pain 52-54

URINARY DISORDERS

1.Yes 2.No 1.Yes 2.No

Chyluria .... Hematuria 55-56

APPETITE

1. Normal.....2.Decrease..... 3.Increase 57

WEIGHT

1. No change..... 2.Increase.....3.Decrease..... 58

PHYSICAL EXAMINATION

1.Not palpable 2.P.D.I. 3.More than 2 inches

Liver 59 60

1.Not palpable 2.P.D.I. 3.$\frac{1}{2}$ to Umbil 4.To Umbil 5.Below Umbil

Spleen 61

1.None 2.Slight 3.Severe

Anemia 62

Jaundice 63

Ascites 64

Lympha. 65

Location of pain or distress: 1 2 3 4 66 67

1.Good 2.Fair 3.Poor

Physic.Devel. ....

Nutrition ....

DIET

1.Part 2.Entire (Vegetable 1. None 2.Some 3.Plenty 68-69

Hicua .... Other Rice 70-71

1.None 2.Some 3.Plenty 1.None 2.Some 3.Plenty

Other fish .... Other meat..... ....

GENERAL LEVEL (To be determined by physician)

1. Less than average.... 2.Average.... 3. Better than average.... 72

MULTIPLICITY OF INFECTION

Helminths..... 73

Protozoa..... 74

Total 75

NOTE: All figures on the Survey Sheet are for the purpose of coding for future tabulation. When making entries, no attention is required by an examiner.

Figure 4. Epidemiological Questionnaire

Besides information on these points the surveys obtain accurate data for use by the Section of Public Health and Welfare, GHQ, SCAP, in connection with the multitudinous facets of their public health problem and it is hoped to furnish information that will be useful in determining the feasibility and the economic desirability of attempting ultimately to eliminate helminths from the Japanese population. Perhaps in the absence of clinical symptoms of hookworm disease, for example, such a program may not be justified. Such a conclusion is incredulous in an area where helmintn prevalencies for as many as 3-5 helminth range 50-90 per cent, but ill effects from these infections even in multiplicity are not readily detectable and may prove to be limited to a small proportion of the population. It is most difficult to ascertain suitable standards for "normalcy" in a population so heavily parasitized and perhaps any general effects are masked by this lack of a group of non-parasitized controls.

It is obvious that, while the present surveys may provide some answers to questions such as those noted above, they will merely serve to delineate many other questions and to point to possible lines of approach.

Background - To date the summaries of approximately 20 Japanese parasitological surveys have been translated. They represent only a portion of such investigations but since most of these were published between 1935-1940, they should be fairly representative of the type and quality of the survey work done during the decade immediately preceding World War II. Some of the data in these articles merit further discussions. Nishio et al (7a) examined 1385 children in four different primary schools in Fukuoka Prefecture - they had received vermifuge from one to four years previously. The infection rates of ascaris, whipworm and hookworm were 26, 8 and 1.2 percent respectively. Katayama (7b) surveyed school children in Kumamoto Prefecture in 1937 and 1938. The average findings for his two projects, which showed considerable differences, were approximately 55 percent for ascaris, 32 percent for whipworm and 12 percent for hookworm. Matsuda, Maeda and others made numerous small surveys in the Osaka and Hiroshima areas, including islands adjacent to the Hiroshima coast. The findings for their surveys varied greatly. In the case of ascaris, they ranged from 10-80 percent with an average of 55-60 percent. In the case of whipworm the rate was between 30-35 percent. In the Hiroshima surveys, the hookworm rate was low, 2 percent, while at Osaka they obtained a rate of 15 percent. The above surveys are summarized by Matsuda (8). This author also obtained pinworm rates of about 43 percent by means of anal swabs. Cellophane, cotton and gauze were all used as swabs. Examination of 111,407 individuals in Aichi Prefecture were reported by Sato (9) in 1940. In this large number 26 harbored ascaris and 7 and 0.5 percent harbored whipworm and hookworm respectively. Low rates were reported for other helminths; protozoa were not diagnosed.

From 1933-1937, Yoshida (10) and others examined 18,071 individuals at an Osaka clinic. Only rates for ascaris and hookworm were given, and these were curiously low, averaging 16 and 2 percent respectively. In Shimane Prefecture Iwata et al (11) 1940, reported rates of 11, 37 and 9 percent for ascaris, whipworm and hookworm in 457 individuals. These authors also recovered pinworm from 30-35 percent by the use of anal swabs. In Hyogo Prefecture in 1940, Aihara (12) reported helminth rates for 272 individuals; ascaris was found to be 19, whipworm 2 and for hookworm, 21 percent.

A comparison of these surveys with the present figures reported herein reveals a considerable discrepancy (Tables X to XV). A critical comparison with the Japanese data is not possible because of a number of factors. The chief points include the omission of data on protozoa, the absence of uniform technics and (in many instances) of well-trained technicians, the utilization of school children almost to the exclusion of other age groups, and usually the reporting of the incidence of parasites only. Even with direct smears in the hands of expertly trained technicians one could not hope to secure results comparable to the concentration technique. An example of this occurs in Figure 5 which summarizes our findings with direct smears in comparison with MGL and AMS III concentration techniques. The direct smears fall far out of the class of the concentration techniques with the worms and the discrepancy becomes even more marked among the protozoa. The incidence on the direct smears is probably higher than usual because only carefully trained technicians made these observations.

Present Status of Analysis of Results - In the presentation that follows only the more important parasites have been selected and tabulated. Many of the data have not been analyzed. In the overall picture the incidence of parasitism is extremely high, with 90 to 95 percent of the individuals examined being infected with one or more helminths, while somewhat less than half were infected with one or more protozoa. The protozoan rates serve as a better index of contamination of food or water than the worms which are more nearly universally present. However, the incidence of protozoa taken together with the density index of the parasitic worms is sufficient to provide a quick picture of a given area since both are higher when sanitary conditions are poorer. A summary of the incidence and density by species of parasite is presented in Figures 5 and 6. One of the most striking situations occurs in Kofu in Yamanashi Prefecture where the incidence of both worms and protozoa is high. There whipworm is more prevalent than ascaris which is usually regarded as the most widely distributed worm in Japan.

INTESTINAL PARASITES

COMPARISON OF CONCENTRATION TECHNIQUES WITH DIRECT SMEARS

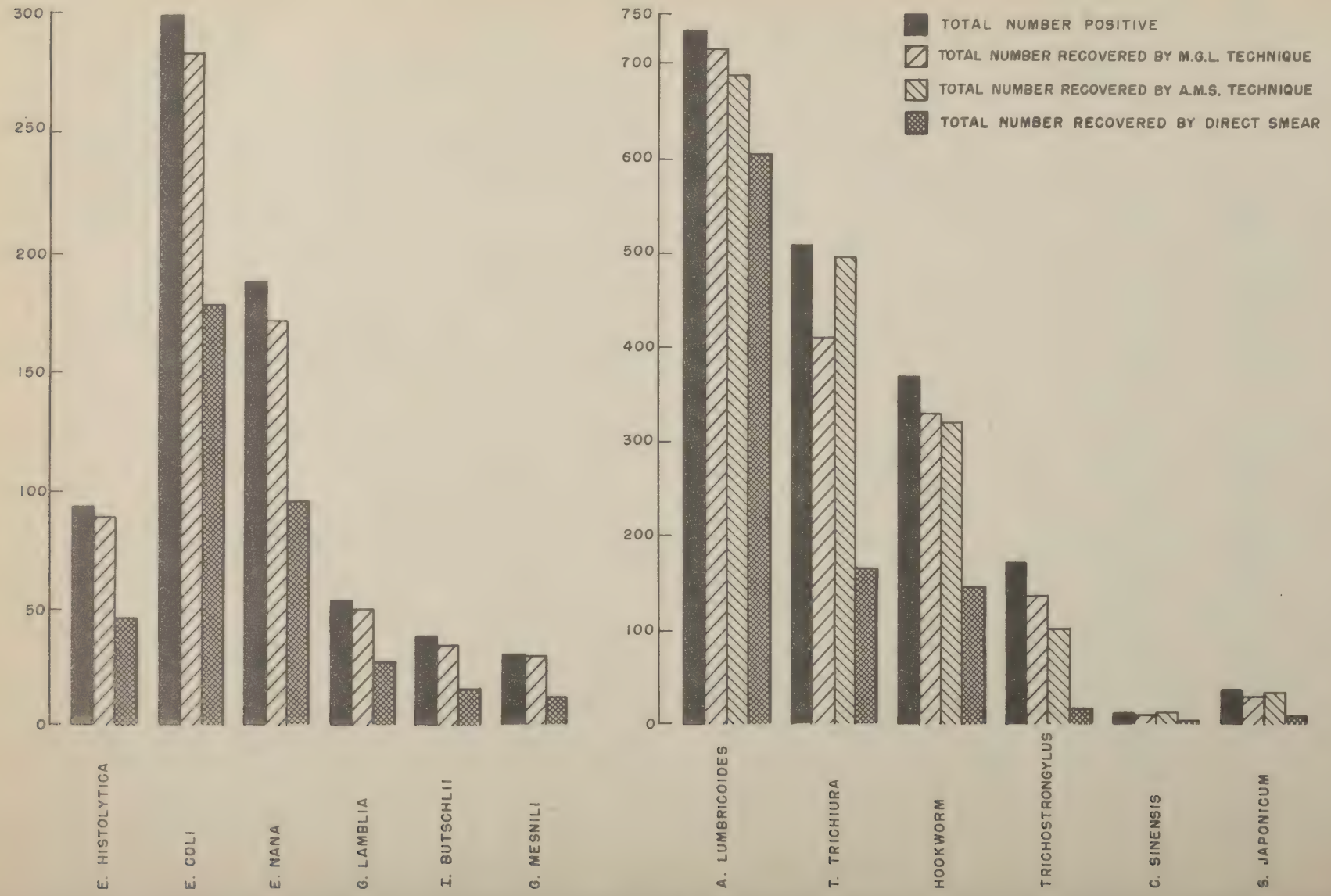
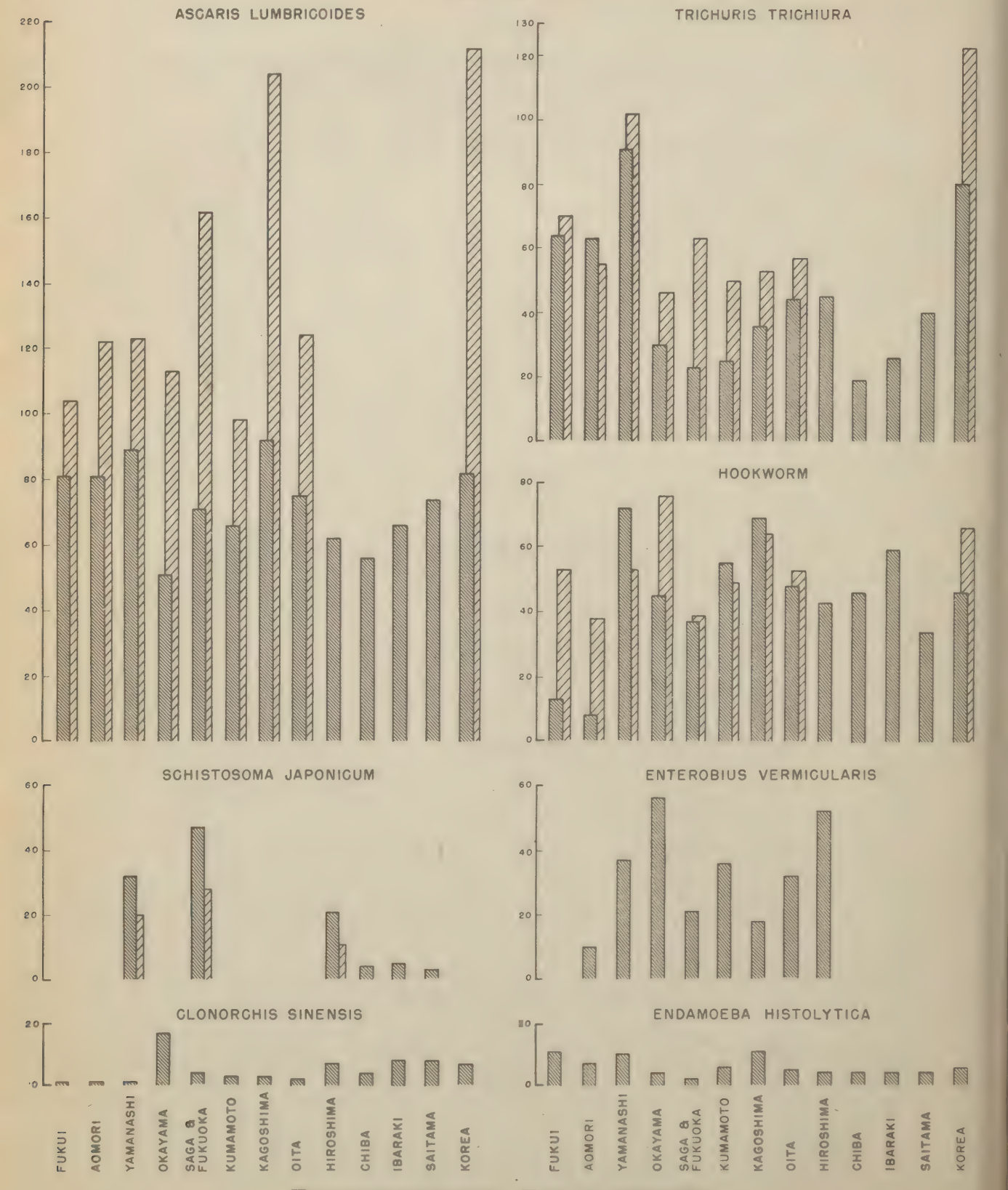


FIG. 5

INFECTION RATES AND PARASITE DENSITIES

IN VARIOUS EPIDEMIOLOGICAL SURVEYS

1947-48



In general the incidence and density of the parasitism is probably due to a combination of interesting factors which include: the length of time that the night soil was stored, location of the population group studied (for some worms such as ascaris appear to be more prevalent in the hillside communities), the presence of door-yard gardens and whether or not they are fertilized by fresh night soil, the frequency with which fields, wells or houses are flooded, the type of farming, and others. These various factors are in the process of being evaluated at the present time.

The use of the parasite density index is shown in schistosomiasis where the results on four of the five known endemic areas are presented (see Fig. 6). In recent years Yamanashi has usually been considered the worst schistosomiasis region in Japan. However, only 32 percent were infected while in Saga and Fukuoka Prefectures it was 47 perccent and in Hiroshima only 21 percent. The Tone River Valley area of Chiba, Ibaraki and Saitama Prefectures runs between 4 and 5 percent and the density index is less than five, which means a light infection. However, Yamanashi had a density index of 20 while Fukuoka and Saga jumped to 28. This means that not only were more people infected there with schistosomiasis but also they were more heavily infected in those villages that were surveyed.

In a similar way the infection with the other worms can be analyzed. It is interesting and perhaps significant that the whipworm density is universally high in Japan and both the percent and the density factors approach those of ascaris - a situation not previously recognized. This is certainly true as it is well recognized that ascaris produces greater numbers of eggs per worm than whipworm and the figures are not corrected to show the approximate number of worms present.

Malaria was not emphasized in most of the epidemiological surveys since the areas were pretty sharply delineated. However, this was not the case with filariasis. In Kyushu two groups of approximately 100 each were examined in areas where filariasis was supposed to exist; 112 people from Yamanishi in Kumamoto Prefecture and 108 from Sesekushi-mura in Kagoshima Prefecture. No filariasis bancrofti was found in the first but 21 or 19.4 percent was detected by the modified Knott Technique (13) in the second. It should be noted that there was no apparent correlation between the positive cases and clinical disease. The incidence seems to be unusually high.

There has been no attempt at this time to analyze and summarize these surveys in detail as they have all been presented previously in the form of preliminary reports. The figures of incidence and density speak for themselves and point to the universality of worms and protozoa which exists here in the Japanese. It seems inevitable that the overall health of the individuals must be deleteriously affected in the case of the heavy, multiple infections with a high parasite index.

(3) A Survey for Intestinal and Blood Parasites in South Koreans - In August 1948 a survey was undertaken to examine about 400 Americans and 1600 - 2000 South Koreans. It was felt that a comprehensive survey of persons from various regions in South Korea would furnish a more accurate overall picture of both the incidence and density of intestinal protozoa and worms than could be obtained from hospital admissions or a more localized survey (14, 15, 16). Due to unforeseen difficulties only 915 Koreans were examined from Seoul, Anyang, Chunch'on, Taejon, Kwanju, Mok-Po, Yongch'on, Pusan and Cheju-Do (Fig. 7). The techniques used in the survey were similar to those used in Japan, except that the physical examinations were conducted by Korean physicians. The samples collected were transported to Tokyo for examination by the same technicians used in the Japanese surveys.

As in the surveys of Japanese (See Tables X to XV) the incidence of intestinal parasites was high. Reference to Table XVI reveals that 871 of the 915, or 95.1 percent, were parasitized. Of these 94.7 percent carried helminths and 35.6 percent harbored protozoa. In the case of both ascaris and whipworm, the parasite density index was higher than any encountered in Japan (see Fig. 6). This supports the comments made by many workers to the effect that the Koreans as a group are unusually heavily parasitized. It is interesting to note that the parasite density index for hookworm was less than in some areas in Japan. As in many areas of Japan ascaris and whipworm were present in 82.1 and 80.0 percent respectively. Hookworm averaged 45.5 percent and Trichostrongylus sp 23.8 percent. The rates on the latter varied from 4.9 percent in Cheju-Do to 52.3 percent in Pusan. Over 6 percent of the population examined carried Clonorchis meaning that considerable quantities of raw fish are being consumed. The rates on clonorchiasis ranged between none on Cheju-Do and Chunch'on to 36.9 percent at Yongch'on near Taegu. Metagonimus averaged 1.7 percent; it was found only in Yongch'on, Pusan and Cheju-Do. As noted previously only 35.6 percent of the 915 South Koreans who were examined were found to harbor protozoan parasites. This compares with rates in various parts of Japan. It is rather surprising that the overall rates were not higher as the water supply is far from satisfactory and there can be little doubt of food contamination since many individuals have little concept of cleanliness. Furthermore, flies are present in great quantity.

Endamoeba histolytica was present in only 5.5 percent of the population examined while Endamoeba coli occurred in 28.5 percent and Endolimax nana in only 9.1 percent. Giardia lamblia, a possible pathogenic protozoan, was present in 3.6 percent of the population while the remaining data are presented in Table XVI. Although the rates in Seoul for E. histolytica were not high (4.8 percent) and they remained near

Table X. Incidence of Intestinal Parasites in Yamanashi Prefecture

City or Village Hamlet	Kissawa		Kofu City		Otsuka		Hikawa		Sancho		Futakawa		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
No. Persons Examined	506		501		532		500		508		508		3,055	
Number with Parasites	501	99.0	498	99.4	529	99.4	499	99.8	507	99.8	507	99.8	3,041	99.5
Number with Helminths	501	99.0	497	99.2	529	99.4	499	99.8	507	99.8	507	99.8	3,040	99.5
Number with Protozoa	343	67.8	326	65.1	384	72.2	358	71.6	316	62.2	269	53.0	1,996	65.3
A. lumbricoides	477	94.3	440	87.8	433	81.4	473	94.6	461	90.7	430	84.6	2,714	88.8
T. trichiura	487	96.2	467	93.2	475	89.3	478	95.6	440	86.6	450	88.6	2,797	91.6
Hookworm	368	72.7	298	59.5	342	64.3	367	73.4	404	79.5	413	81.3	2,192	71.8
Trichostrongylus sp.	126	24.9	225	44.9	337	63.3	84	16.8	249	49.0	334	65.7	1,355	44.4
S. japonicum	152	30.0	17	3.4	146	27.4	1	0.2	335	65.9	328	64.6	979	32.0
C. sinensis	1	0.2	3	0.6	1	0.2	3	0.6	6	1.2	1	0.2	15	0.5
E. histolytica	80	15.8	62	12.4	56	10.5	64	12.8	34	6.7	19	3.7	315	10.3
E. coli	226	44.7	207	41.3	265	49.8	266	53.2	175	34.4	172	33.9	1,311	42.9
E. nana	106	20.9	108	21.6	130	24.4	155	31.0	87	17.1	87	17.1	673	22.0
G. lamblia	14	2.8	23	4.6	26	4.9	23	4.6	30	5.9	37	7.3	155	5.0

Table XI. Incidence of Intestinal Parasitism in Fukui Prefecture

City or Village Hamlet	Kamiyama		Fukui City		Naruka		Shimo-Shii		Goryogashima		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
No. Persons Examined	394		419		391		45		47		1296	
Number with Parasites	374	94.9	385	91.9	373	95.4	45	100	36	76.6	1213	93.6
No. with Helminths	369	93.7	364	86.9	356	91.0	44	97.8	31	66.0	1164	89.8
No. with Protozoa	160	40.6	178	42.5	246	62.9	25	55.6	17	36.2	626	48.3
A. lumbricoides	342	86.8	320	76.4	320	81.8	42	93.3	25	53.2	1049	80.9
T. trichiura	286	72.6	242	57.8	243	62.1	38	84.4	16	34.0	825	63.7
Hookworm	80	20.3	56	13.4	21	5.4	7	15.6	3	6.4	167	12.9
Trichostrongylus sp.	16	4.1	22	5.3	6	1.5	1	2.2	0	0.0	45	3.5
S. japonicum	-	-	-	-	-	-	-	-	-	-	-	-
C. sinensis	-	-	-	-	-	-	-	-	-	-	-	-
E. histolytica	25	6.3	34	8.1	75	19.2	9	20.0	4	8.5	147	11.3
E. coli	107	27.2	88	21.0	152	38.9	16	35.6	15	31.9	378	29.2
E. nana	61	15.5	62	14.8	125	32.0	10	22.2	5	10.6	263	20.3
G. lamblia	16	4.1	45	10.7	43	11.0	3	6.7	2	4.3	109	8.4

Table XII. Incidence of Intestinal Parasitism in Aomori Prefecture

City or Village Hamlet	Aomori City		Ominato				Hirosaki				Hachinoe				Total	
			Jogasawa		Isumisawa		Fujishiro		Tsuruda		Nakaebayasni		Minatoku			
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
No. Persons Examined	416		303		108		377		121		137		86		1548	
No. with Parasites	400	96.2	302	99.7	108	100	355	94.2	119	98.3	113	82.5	71	82.6	1468	94.8
No. with Helminths	391	94.0	302	99.7	107	99.1	350	92.8	118	97.5	99	72.3	58	67.4	1425	92.1
No. with Protozoa	190	45.7	181	59.7	70	64.8	121	32.1	37	30.6	64	46.7	38	44.2	701	45.3
A. lumbricoides	344	82.7	269	88.8	92	85.2	295	78.2	112	92.6	92	67.2	49	57.0	1253	80.9
T. trichiura	292	70.2	281	92.7	95	88.0	183	48.5	78	64.5	31	22.6	22	25.6	982	63.4
Hookworm	16	3.9	30	9.9	12	11.1	54	14.3	8	6.6	-	-	1	1.2	121	7.8
Trichostrongylus sp.	156	37.5	62	20.5	22	20.4	252	66.8	100	82.6	2	1.5	1	1.2	595	38.4
S. japonicum																
C. sinensis															7	0.5
E. histolytica	32	7.7	41	13.5	17	15.7	13	3.4	2	1.7	6	4.4	3	3.5	114	7.4
E. coli	124	29.8	137	45.2	49	45.4	78	20.7	15	12.4	47	34 3	30	34 9	480	31.0
E. nana	71	17.1	75	24.8	31	28.8	44	11.7	2	1.7	27	19.7	18	20.9	268	17.3
G. lamblia	29	7.0	14	4.5	16	14.8	22	5.8	16	13 2	5	3.6	4	4.7	106	6.8

Table XIII. Incidence of Intestinal Parasitism in Okayama Prefecture

City or Village Hamlet	Notani		Kojo		Okayama City		Total	
	No.	%	No.	%	No.	%	No.	%
Number Persons Examined	415		407		438		1,260	
Number with Parasites	377	90.8	348	85.5	401	91.6	1,126	89.4
Number with Helminths	364	87.7	331	81.3	384	87.7	1,079	85.6
Number with Protozoa	163	39.3	133	32.7	185	42.2	481	38.2
A. lumbricoides	214	51.6	142	34.9	290	66.2	646	51.3
T. trichiura	113	27.2	92	22.6	170	38.8	375	29.8
Hookworm	284	68.4	137	33.7	151	34.5	572	45.4
Trichostrongylus sp.	9	2.2	4	1.0	9	2.1	22	1.7
S. japonicum	0	0.0	0	0.0	0	0.0	0	0.0
C. sinensis	16	3.9	164	40.3	28	6.4	208	16.5
E. histolytica	19	4.6	8	2.0	24	5.5	51	4.0
E. coli	115	27.7	82	20.1	121	27.6	318	25.2
E. nana	65	15.7	42	10.3	71	16.2	178	14.1
G. lamblia	22	5.3	24	5.9	25	5.7	71	5.6

Table XIV. Incidence of Intestinal Parasitism in Kyushu

Prefecture	Saga & Fukuoka										Ohita					
City or Village Hamlet	Kurume City Nagatoishi		Tosu Anrakuji		Asai Gitoku		Kiyama		Total		Beppu I (hillside)		Beppu II (coast)		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
No. Persons Examined	214		201		201		100		716		229		200		429	
No. with Parasites	203	94.9	192	95.5	180	89.6	99	99.0	674	94.1	221	96.5	184	92.0	405	94.4
No. with Helminths	200	93.5	191	95.0	177	88.1	99	99.0	667	93.2	219	95.6	178	89.0	397	92.5
No. with Protozoa	36	16.8	46	22.9	32	15.9	24	24.0	138	19.3	101	44.1	95	47.5	196	45.7
A. lumbricoides	131	61.2	149	74.1	131	65.2	95	95.0	506	70.7	189	82.5	134	67.0	323	75.3
T. trichiura	25	11.7	29	14.4	43	21.4	65	65.0	162	22.6	116	50.7	74	37.0	190	44.3
Hookworm	65	30.4	62	30.8	72	35.8	65	65.0	264	36.9	136	59.4	69	34.5	205	47.8
Trichostrongylus sp.	6	2.8	0	0.0	1	0.5	7	7.0	14	2.0	21	9.2	6	3.0	27	6.3
S. japonicum	156	72.9	121	60.2	59	29.4	1	1.0	337	47.1	0	0.0	0	0.0	0	0.0
C. sinensis	16	7.5	7	3.5	4	2.0	2	2.0	29	4.1	4	1.7	6	3.0	10	2.3
E. histolytica	1	0.5	3	1.5	7	3.5	2	2.0	13	1.8	11	4.8	12	6.0	23	5.4
E. coli	16	7.5	19	9.5	17	8.5	16	16.0	68	9.5	72	31.4	60	30.0	132	30.8
E. nana	7	3.3	13	6.5	10	5.0	6	6.0	36	5.0	33	14.4	33	16.5	66	15.4
G. lamblia	18	8.4	17	8.5	8	4.0	5	5.0	48	6.7	13	5.7	20	10.0	33	7.7

Prefecture	Kagoshima						Kumamoto								
City or Village Hamlet	Kagoshima City		Ishiki Kamiishiki		Total		Kumamoto		Ohshima		Yamanishi		Total		Total for Kyushu
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
No. Persons Examined	203		202		405		204		207		112		523		2,073	
No. with Parasites	200	98.5	202	100	402	99.3	191	93.6	180	87.0	101	90.2	472	90.2	1,953	94.2
No. with Helminths	199	98.0	202	100	401	99.0	179	87.7	176	85.0	100	89.3	455	87.0	1,920	92.4
No. with Protozoa	100	49.3	64	31.7	164	40.5	95	46.6	57	27.5	38	33.9	190	36.3	688	33.2
A. lumbricoides	177	87.2	197	97.5	374	92.3	133	65.2	124	59.9	86	76.8	343	65.6	1,546	74.6
T. trichiura	89	43.8	58	28.7	147	36.3	66	32.4	50	24.2	15	13.4	131	25.0	630	30.4
Hookworm	126	62.1	155	76.7	281	69.4	119	58.3	109	52.7	62	15.4	290	55.4	1,040	50.2
Trichostrongylus sp.	5	2.5	10	5.0	15	3.7	4	2.0	3	1.4	4	3.4	11	2.1	67	3.2
S. japonicum	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	337	16.3
C. sinensis	12	5.9	1	0.5	13	3.2	7	3.4	7	3.4	2	1.8	16	3.1	68	3.3
E. histolytica	30	14.8	15	7.4	45	11.1	12	5.9	12	5.8	5	4.5	29	5.5	110	5.3
E. coli	69	34.0	34	16.8	103	25.4	58	28.4	28	13.5	23	20.5	109	20.8	412	19.9
E. nana	39	19.2	27	13.4	66	16.3	46	22.5	20	9.7	16	14.3	82	15.7	250	12.1
G. lamblia	7	3.4	6	3.0	13	3.2	13	6.4	10	4.8	7	6.3	30	5.7	124	6.0

Table XV Incidence of Intestinal Parasitism in Saitama and Ibaraki Prefecture

Prefecture	Saitama											
City or Village	Soka		Hikonari		Waseda		Towa		Miwanoe		Total	
Hamlet							Togasaki					
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Number Persons Examined	210		103		101		100		106		620	
Number with Parasites	184	87.6	100	97.0	95	94.0	94	94.0	95	89.6	568	91.6
Number with Helminths	176	83.8	100	97.0	95	94.0	93	93.0	94	88.6	558	90.0
Number with Protozoa	71	33.8	39	37.8	20	19.8	43	43.0	27	25.4	200	32.3
A. lumbricoides	138	65.7	84	81.5	84	83.1	83	83.0	71	66.8	460	74.2
T. trichiura	77	36.6	56	54.3	44	43.5	45	45.0	26	24.5	248	40.0
Hookworm	39	18.5	52	50.4	24	23.7	39	39.0	54	50.9	208	33.5
Trichostrongylus sp.	36	17.1	41	39.8	33	32.6	20	20.0	16	15.0	146	23.5
S. japonicum	-	-	1	0.9	-	-	14	14.0	1	0.9	16	2.6
C. sinensis	13	6.1	19	18.4	4	3.9	11	11.0	1	0.9	48	7.7
E. histolytica	12	5.7	2	1.9	4	3.9	6	6.0	3	2.8	27	4.4
E. coli	42	20.0	25	24.2	14	13.8	29	29.0	17	16.0	127	20.5
E. nana	24	11.4	23	22.3	8	7.9	12	12.0	15	14.1	82	13.2
G. lamblia	8	3.8	4	3.8	1	0.9	8	8.0	3	2.8	24	3.9

Prefecture	Ibaraki											
City or Village	Nagasu		Nanago		Ono		Koya		Inatoi			
Hamlet	Kimagase		Yahagi		Okashiwa		Koya		Nonoi		Togashiwa	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
No. Persons Examined	129		128		120		129		50		123	
Number with Parasites	125	96.9	126	98.4	101	84.2	117	90.7	46	92.0	108	87.8
Number with Helminths	124	96.1	126	98.4	96	80.0	107	82.9	42	84.0	105	85.3
Number with Protozoa	40	31.0	35	27.3	33	27.5	46	35.6	26	52.0	38	30.8
A. lumbricoides	95	73.6	98	76.5	64	53.0	80	62.0	34	68.0	75	60.9
T. trichiura	34	26.3	46	35.9	16	13.3	29	22.4	7	14.0	11	8.9
Hookworm	102	79.0	108	84.3	53	44.2	56	43.4	28	56.0	54	43.9
Trichostrongylus sp.	8	6.2	11	8.5	2	1.6	2	1.5	2	4.0	8	6.5
S. japonicum	-	-	-	-	2	1.6	13	10.1	4	8.0	13	10.5
C. sinensis	12	9.3	16	12.5	8	6.6	2	1.5	3	6.0	14	11.3
E. histolytica	8	6.2	-	-	4	3.3	2	1.5	2	4.0	7	5.6
E. coli	29	22.4	23	17.9	22	18.3	32	24.8	18	36.0	31	24.2
E. nana	21	16.2	18	14.0	9	7.5	19	14.7	14	28.0	20	16.2
G. lamblia	4	3.1	5	3.9	8	6.6	9	6.9	1	2.0	1	0.8

Prefecture	Ibaraki (Cont.)									
City or Village	Toride		Omonma				Manaita			
Hamlet	Yoshida		Minami		Todai		Daitoku-		Total	
							Nabeko Shinden			
	No.	%	No.	%	No.	%	No.	%	No.	%
Number Persons Examined	126		65		65		130		1,065	
Number with Parasites	119	94.4	65	100	62	95.3	126	96.9	995	93.4
Number with Helminths	119	94.4	64	98.4	62	95.3	124	95.3	969	90.9
Number with Protozoa	34	26.9	26	40.0	15	23.0	51	39.2	344	32.3
A. lumbricoides	77	61.1	54	83.0	47	72.3	84	64.6	708	66.4
T. trichiura	28	22.2	28	43.0	38	58.4	45	34.6	282	26.4
Hookworm	87	69.0	20	30.7	30	46.1	91	70.0	629	59.0
Trichostrongylus sp.	49	38.8	43	66.1	58	86.1	72	55.3	255	23.9
S. japonicum	6	4.7	3	4.6	3	4.6	11	8.4	55	5.1
C. sinensis	8	6.3	7	10.7	9	13.8	9	6.9	88	8.2
E. histolytica	4	3.1	4	6.1	4	6.1	5	3.8	40	3.7
E. coli	19	15.0	19	29.2	9	13.8	31	23.8	233	21.8
E. nana	18	14.2	19	29.2	5	7.6	23	17.6	166	15.5
G. lamblia	4	3.1	1	1.5	-	-	4	3.0	37	3.4

Table XV. Continued - Incidence of Intestinal Parasitism in Chiba

Prefecture	Chiba													
City or Village	Abiko		Tomisei		Tanaka		Kohoku		Fukuda		Sakura		Total	
Hamlet	Aoyama Shibasaki				Omura				Takane					
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
No. Persons Examined	117		104		148		149		100		222		840	
Number with Parasites	97	82.9	94	90.4	128	86.5	129	86.6	90	90.0	193	86.9	731	87.0
Number with Helminths	94	80.3	86	82.9	126	85.1	125	89.8	89	89.0	182	81.9	702	83.5
Number with Protozoa	27	23.1	46	44.2	45	30.4	50	33.6	8	8.0	87	39.1	263	31.3
A. lumbricoides	59	50.4	65	62.5	83	56.1	74	49.6	70	70.0	118	53.1	469	55.8
T. trichiura	6	5.1	32	30.7	38	25.7	33	22.1	7	7.0	45	20.2	161	19.1
Hookworm	47	40.1	44	42.3	74	50.0	93	62.4	41	41.0	85	38.2	384	45.7
Trichostrongylus sp.	9	7.7	5	4.8	2	1.3	15	10.1	3	3.0	73	32.8	107	12.7
S. japonicum	13	11.1	-	-	18	12.1	1	0.7	-	-	-	-	32	3.8
C. sinensis	4	3.4	2	1.9	4	2.7	7	4.7	5	5.0	12	5.4	34	4.0
E. histolytica	2	1.7	9	8.6	9	6.1	6	4.0	-	-	10	4.5	36	4.2
E. coli	19	16.2	27	25.9	26	17.5	31	20.8	3	3.0	54	24.3	160	19.0
E. nana	3	2.5	17	16.3	22	14.8	21	14.1	5	5.0	27	12.1	95	11.3
G. lamblia	6	5.1	9	8.6	4	2.7	7	4.7	1	1.0	17	7.6	44	5.2

Table XVI. Summary of Incidence of Intestinal Parasites by Community in South Korea

Community	Seoul		Anyang		Chunch'on		Taejon		Kwangju	
	No.	%	No.	%	No.	%	No.	%	No.	%
No. of Persons Examined	167		70		262		80		45	
Number with Parasites	159	95.2	68	97.1	250	95.4	70	87.5	43	95.6
Number with Helminths	159	95.2	66	94.3	250	95.4	68	85.0	43	95.6
Number with Protozoa	55	32.9	28	40.0	93	35.5	26	32.5	18	40.0
A. lumbricoides	136	81.4	61	87.1	232	88.6	66	82.5	35	77.8
T. trichiura	146	86.8	50	71.4	193	73.3	49	61.3	38	84.4
Hookworm	65	38.9	32	45.7	126	48.1	25	31.3	21	46.7
Trichostrongylus sp.	59	35.3	14	20.0	22	8.4	10	12.5	7	15.6
Taenia sp.	2	1.2	-	-	4	1.5	-	-	1	2.2
C. sinensis	9	5.4	1	1.4	-	-	3	3.8	4	8.9
E. histolytica	8	4.8	4	5.7	13	5.0	1	1.3	5	11.1
E. coli	44	26.4	22	31.4	68	26.0	19	23.8	15	33.3
E. nana	19	8.4	5	7.1	33	12.6	8	10.0	2	4.4
G. lamblia	6	3.6	-	-	17	6.5	2	2.5	4	8.9

Community	Mok-Po		Pusan		Taegu		Cheju-Do		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%
No. of Persons Examined	44		93		73		81		915	
Number with Parasites	40	90.1	92	98.9	72	98.6	77	95.1	871	95.1
Number with Helminths	40	90.1	92	98.9	72	98.6	77	95.1	867	94.7
Number with Protozoa	15	34.1	30	32.3	24	32.9	37	45.7	326	35.6
A. lumbricoides	33	75.0	81	87.1	56	76.7	52	64.2	752	82.1
T. trichiura	31	70.5	85	91.4	68	93.2	72	88.9	732	80.0
Hookworm	19	43.2	70	75.3	39	53.4	20	24.7	417	45.5
Trichostrongylus sp.	21	47.4	49	53.7	32	43.8	4	4.9	218	23.8
Taenia sp.	1	2.3	3	3.2	1	1.4	14	17.3	26	2.8
C. sinensis	1	2.3	16	17.2	27	37.0	-	-	61	6.6
E. histolytica	2	4.6	4	4.3	6	8.2	8	9.9	51	5.5
E. coli	14	31.8	25	26.9	20	27.4	34	42.0	261	28.5
E. nana	3	6.8	6	6.5	6	8.2	2	2.5	84	9.1
G. lamblia	-	-	1	1.1	2	2.7	1	1.2	33	3.6


LOCALITIES SURVEYED FOR PARASITES
SOUTH KOREA, AUGUST 1948
MANCHURIA
U.S.S.R.
HAMGYONG-PUKTO
NANAM
SINUIJU
PYONGAN-PUKTO
HAMGYONG-NAMDO
N
HAMHUNG
PYONGAN-NAMDO
PYONGYANG
LEGEND
INTERNATIONAL BOUNDARY
PROVINCIAL BOUNDARY
NATIONAL CAPITAL
PROVINCIAL CAPITAL
LOCALITIES UNDERLINED SURVEYED FOR PARASITES AUGUST 1948.
38°
KWANGHAE-DO
HAEJU
KANGWON-DO
KYONGGI-DO
NORTH KOREA (U.S.S.R. ZONE)
SOUTH KOREA (U.S. ZONE)
38°
SEOUL
CHUNCHON
ANYANG
CHUNG-CHONG-NAMDO
CHUNG-CHONG-PUKTO
CHONGJU
TAEJON
KYONGSANG-PUKTO
CHOLLA-PUKTO
CHONJU
YONGCHON
TAEGU
KWANGJU
KYONGSANG-NAMDO
MOKPO
CHOLLA-NAMDO
PUSAN
SCALE
MILES
0
50
100
150
CHEJU-DO
TSUSHIMA
JAPAN
FIG. 7

this figure at Anyang and Chunch'on, they dropped to a surprising and almost unbelievable low of 1.25 percent at Taejon. The explanation of this is not clear although the water supply was reported to be more satisfactory there than in many other areas. In Kwanju it reached 11.1 percent, a figure which was approached only at Yongch'on and Cheju-Do respectively. It is extremely difficult to explain the comparatively "normal" incidence of E. histolytica when the habits of the Koreans as a whole are considered.

The malaria rates in troops in Southern Korea were sufficiently high to suggest a large reservoir of infections among the Koreans. Attempts were made to secure a representative picture but technical difficulties were encountered. Out of 534 Koreans examined approximately 3 percent yielded positive malaria smears; these were all Plasmodium vivax.

It was hoped to examine a considerable number of blood samples for microfilariae. Only 23 were examined from Southern Korea proper and 35 from Cheju-Do. Two, or 5.7 percent, of these were positive for Wuchereria malayi. The presence of this species in Korea is believed to constitute a new distribution record. In surveys for filariasis in Japan, which have been limited chiefly to Kyushu Island, only W. bancrofti has been encountered. Of the two individuals who were found to be infected with W. malayi only one reported foreign travel, to Japan.

In conclusion it should be emphasized that Koreans have both a high incidence of parasitism and a high parasite density index. However, it should be pointed out that this applied primarily to the worm burden and not to protozoa. In this connection it should be noted that it was not possible to substantiate the high incidence of amebiasis reported by some workers.

(4) Parasitological Findings for Kozu-Jima - Thirty-seven stools and ten blood specimens were received from the small island of Kozu which is located off the coast of Honshu in the vicinity of Tokyo. Ascaris was the dominant helminth - eggs of this worm were found in 32 of the 37 specimens; only one specimen contained whipworm eggs and hookworm eggs were present in 11. There were no cases of amebiasis but other protozoa were found of which there was a preponderancy of E. coli. Examinations for filariasis by means of Knott's concentration technique were all negative.

(5) Summary of Blood Parasites in Birds - During the course of the studies on Japanese B encephalitis it seemed desirable to secure information on the blood parasites of birds. This project has not yet been completed. The information compiled to date indicates that 80 species totaling about 4735 specimens have been examined for blood parasites primarily by Japanese workers. Four groups of parasites, Plasmodium (malaria), Haemoproteus, Leucocytozoan and Trypanosoma were encountered. The incidence is not high. The data are summarized in Table XVII.

(6) Survey for Schistosomiasis at Itazuke - At the request of the Surgeon's Office of the 315th Composite Wing a lake was examined to determine whether or not it was safe for swimming. This study was indicated in view of the proximity of this area to the Tosu-Kurume schistosomiasis region in Fukuoka and Saga Prefectures. However, no Oncomelania snails were found and the clay and sand bottom, general ecological conditions, altitude and remoteness of paddies virtually eliminated all danger from this disease.

Studies on Santonin and Hexylresorcinol -

(1) On the Efficacy of Santonin - At the request of the Japanese a follow-up program on the stools of persons treated with Santonin was set up. One month following treatment stools were examined of persons previously positive for ascaris. At the end of the second month another stool was examined. As might be expected the results clearly indicate the ineffectiveness of Santonin as an ascaricide in the dosages used. It was forecast that the drug would be ineffective in the concentrations used and the narrow margin of safety between the more efficacious dose and the toxic dose was also recognized. It should be pointed out that the treatment, dosage, etc. was entirely in Japanese hands and all the members of the laboratory did was to examine the stools.

(2) On the Efficacy of Japanese Hexylresorcinol - The Section of Medical Zoology was asked by the Public Health and Welfare of SCAP to participate in a drug assay program for testing hexylresorcinol manufactured by various Japanese drug companies. During three months, 200 stools from each of four villages (Soka in Saitama Prefecture, Sakura in Chiba and Akatsuka and Shimura in Tokyo Prefectures) were examined. Approximately 90 individuals in each of these villages were examined again following treatment by prefectural officials, and the results of the two examinations were compared. Since the selection of individuals for this study was based upon the presence of ascaris alone the occurrence of whipworm and hookworm was coincidental, with the result that only small numbers infected with the latter

two species were secured. This was most unfortunate since hexylresorcinol is also known to be quite effective against these species. The following information was obtained from the post-treatment findings: individuals which became negative, and those in which the egg count either decreased, remained unchanged, or increased. There was considerable difference in the efficacy of treatment achieved in the four villages. In Sakura and Akatsuka 53 and 49 percent of persons positive for ascaris before treatment were negative after treatment, and in Soka and Shimura only 24 and 29 percent became negative. The degree of efficacy indicated by these figures is modified somewhat by other findings such as a decrease, increase or no change at all in the egg count. These four categories were reduced to one by considering the parasite density index for each village before and after treatment, and by determining the reduction of the index. The extent of this reduction in the several villages was as follows: Sakura, 69 percent; Akatsuka, 53 percent; Soka, 48 percent; Shimura, 33 percent. The efficacy of treatment then appears to have been about twice as good in Sakura as in Shimura. The results in Akatsuka and Soka were average with an apparent reduction of the worm burden of about 50 percent. It was later disclosed that the drug was not administered at the same time to the various test group. Consequently accurate comparisons could not be made. Furthermore, a saline purge was not given. These and other uncertainties of procedure cast considerable doubt on the value of the overall survey.

Table XVII. Summary of Blood Parasites of Birds in Japan

Order	Number of Species Examined	Approximate No. of Specimens Exam'd	Number of Species Infected With: Plasmodium	Haemoproteus	Leucocytozoan	Trypanosoma
Passeres (sparrow)	59	4500	27	22	14	10
Halcyones (kingfisher)	1	2	0	0	0	0
Pici (wryneck)	1	6	0	1	0	0
Striges (owl)	3	17	0	1	1	1
Accipitres (hawk)	2	3	0	0	1	0
Gressores (heron)	2	5	0	2	0	0
Anseres (duck)	2	3	0	0	0	0
Columbae (dove)	5	150	1	1	1	1
Limicolae (snipe)	2	27	1	1	1	0
Galli (pheasant, quail)	3	20	0	0	0	0
Total	80	4733	29	28	18	12

Collection and Distribution of Parasitological Materials - During the calendar year approximately 112,500 snails were collected from various parts of Japan. These were known or were suspected of serving as intermediate hosts of various parasitic worms infecting man. All of the snails were used in experiments performed at this laboratory, Kofu, the Japanese National Institute of Health, or were forwarded through the Army Medical Department Research and Graduate School to various research agencies in the United States. About 31,000 ml of preserved mixed protozoa and helminth ova were collected from survey areas and from patients. This material contained all the common protozoa and helminth ova, but is of special value because of the large number of ova present. Much of this material was sent to the Distributing Center for Parasitological specimens for teaching and research purposes. (See Table XVIII). A strain of Trypanosoma gambiense, obtained by the Section through the Japanese National Institute of Health was maintained during 1948 by animal passage in white mice.

Table XVIII. Collection and Distribution of Parasitological Materials

Specimens Received	Items	Milliliters of Material	Recipients
Snails - O. nosophora	112,500		
Formalized feces with:			
S. japonicum and other helminth ova		18,000	
C. sinensis and other helminth ova		3,500	
Hookworm and other helminth ova		2,000	
Strongyloides larvae (rhabditiform) and mixed protozoan cysts		3,000	
Taenia sp. ova		425	
Metagonimus sp. and other helminth ova		2,000	
Trichostrongylus sp. ova		250	
Paragonimus westermani ova		200	
Rhabditis hominis - larvae and adults		400	
Echinochasmus perfoliatus ova		200	
E. histolytica cysts		400	
Total	112,500	31,455	
Specimens Shipped			
Snails - O. nosophora	20,300		NIH (US), NIH (Jap), AMDR&GS
Teaching Kit	19	90	332nd Station Hospital
Teaching Kit	12	60	172nd Station Hospital
Preserved helminth ova and protozoan cysts	37	17,486	AMDR&GS
Teaching Kit	10	280	28th Station Hospital
Teaching Kit	38	180	49th General Hospital Annex
Preserved helminth ova and protozoan cysts	1	130	188th Med. Det. Laboratory
Preserved helminth ova and protozoan cysts	4	400	31st Infantry, 2nd Bn.
Preserved helminth ova and protozoan cysts	1	130	22nd Station Hospital
C. sinensis adults	300		AMDR&GS
S. japonicum adults	300		AMDR&GS
Total	21,022	18,756	

Plans for 1949

As far as it is possible to ascertain at this writing the program of the Section of Medical Zoology will be a continuation and finishing of some of the work initiated in 1947. There are, of course, certain new problems to be considered which are planned while others will be turned up by the epidemiological surveys that remain. The program for the current year as envisioned at the present time is summarized as follows:

Studies on Schistosomiasis - (1) Chemical Control of Schistosomiasis in Yamanashi Prefecture - This cooperative program with the National Institute of Health will be completed during 1949. Extensive field tests on control will be continued in the spring.

(2) Epidemiological Studies on the Snail Host - Monthly collections will be continued to secure additional data on the growth of the snail O. nosophora, and on the infection rates and development of S. japonicum.

(3) Epidemiology of Schistosomiasis - Studies will be carried out in endemic areas to delimit more accurately the boundaries and the extent to which the snail hosts are present and infected. The Numazu area is the one remaining recognized region in which schistosomiasis is endemic that has not been surveyed. Much work also remains to be done in the Tone River Valley.

(4) Snail Control in China - It was hoped to be able to accept the invitation of the Chinese Government to do some work on various diseases, especially the control of schistosomiasis. However, circumstances necessitated a postponement of this mission - perhaps indefinitely.

(5) Immunological Studies on Schistosomiasis - There are two aspects of this problem that merit further consideration. (a) Skin Tests - This problem will be continued to determine whether or not a satisfactory antigen for skin testing can be produced from either adult worms or cercariae. (b) Cross Immunity Studies - With the discovery by the Japanese of an endemic area of bird schistosomiasis on Shinji Lake in Shimane Prefecture it becomes possible to attempt the cross immunity studies outlined last year. These experiments would use bird and human species of schistosomes as the sensitizing agents.

Epidemiology of Trichostrongylus sp. -This parasite has a nigh incidence in certain areas such as Aomori, Yamanashi and the Tone River Valley region. It is particularly fortunate that this new area was discovered in the Tone Valley since it will facilitate the work on experimental animals.

Epidemiological Surveys - Several still remain - Hokkaido, Numazu, Shikoku and part of Hiroshima. With the completion of these over 20,000 Japanese will have been surveyed and data analysis will remain. Undoubtedly this analysis will delineate many more questions than it will answer. Already some of these questions, and the possible approaches to their answer, are apparent.

SEROLOGY SECTION

Routine

During 1948 a total of 63,237 serologic tests were completed. There was no significant monthly variation.

Table I. Serologic Tests

Kahn (Standard)	29,645	Pandy	1,580
Kolmer-Wassermann (Serum	10,400	Colloidal Gold Curve	1,571
Quan. Kolmer-Wassermann (Serum)	430	"Vi" Antibody Tests	1,616
Kolmer-Wassermann (Spinal)	1,580	Standard Slide Microflocculation	2,962
Blood Typing	221	Standard Tube Macroflocculation	206
Cold Agglutinins	230	Standard Wassermann (Serum)	1,008
Rh_o Factor	393	Quantitative Wassermann	93
Rh_o Antibody Titer	245	Standard Wassermann (Spinal)	157
Heterophile Antibody	1,957	Quantitative Slide Flocculation	
Quantitative Kahn	8,082	25% Saline	544
		0.9% Saline	317
		Total Serologic Examinations	63,237

Standard Kahn Tests and Kolmer Wassermanns, as outlined in TM 8-227 are routinely used. For other tests the best available techniques are utilized. As noted in previous Annual Reports, all hospitals in Japan are required to submit sera for testing by this laboratory on any cases shown to be doubtful or positive by the techniques employed in the local laboratories.

Special

Evaluation Studies - In April samples of Kahn positive, doubtful and negative sera were distributed by the 406th to U. S. Army laboratories in Japan, Korea and the Philippine Islands. These sera, accompanied by procedural directions, were to be Kahn-tested by each participating laboratory and the findings reported to the 406th for analysis.

The project had a two-fold design: (1) to determine how much each laboratory's results deviated from those expected and (2) to recommend methods for minimizing these deviations in order to effect greater uniformity throughout the theater.

This survey departed from those conducted in the past, in that all participating laboratories were requested by preliminary and covering letters to carry out the Kahn Tests precisely as indicated in TM 8-227 (Oct. 1946).

Despite this attempt to achieve uniformity of procedure considerable variations in results were noted on termination of the survey.

As has been repeatedly noted in similar surveys in the past there is a definite tendency to over-read results so that "doubtfuls" are reported as "positives" and the readings of the quantitative tests are inordinately high.

It is considered that several factors contribute to this including:

(1) Inexperienced personnel always tend to read high. Paragraph 429 c. (8) of TM 8-227 further contributes to this by indicating that ++++ reactions show definitely visible particles suspended in a transparent or opalescent medium. This is a departure from previous instructions which have required a clear medium for a ++++ reading and tends in general to encourage a higher reading.

(2) The tendency to over-read is also evident in the Quantitative Test in that the officers and/or technicians hesitate to ignore ± or + readings as directed in Paragraph 431 d. TM 8-227.

(3) Clinicians demand a quantitative test on all sera reported as "doubtful" or "positive". Because 0.9% saline is used as the diluent in the standard test and 2.5% saline is used in the quantitative test the latter is more sensitive. It is thus possible to have a standard "doubtful" reading and a report of 40 Kahn units on the same sera. This apparent discrepancy is difficult for the clinician to understand and the easy out is for the laboratory officer to up-grade all standard readings to produce an apparent conformity.

(4) As has been repeatedly noted by all individuals conducting surveys of this type there continues to exist a tendency to adjust the serological technique to the routine of the laboratory rather than the converse.

(5) Finally the inexperienced officer or technician is always "afraid he will miss a positive" rather than being motivated by an intent not to suggest an erroneous diagnosis. The term "diagnosis" is properly used since a single laboratory report is often sufficient to precipitate treatment of the unfortunate individual by the ward physician.

Following the survey the various local hospital laboratories were visited to specifically observe the reasons for variations in the results obtained. At other times during the year reports of difficulties with reagents or procedures were investigated.

Evaluation of Japanese-Performed Complement-Fixation Tests for Syphilis - In March a survey sponsored by the Welfare Ministry of the Japanese Government was carried out. This study attempted to assay the relative merits of the complement-fixation tests for syphilis currently employed by Japanese laboratories. For purposes of comparison this laboratory was asked to perform the Kolmer-Wassermann Test.

The remarks which follow represent the writer's unofficial reactions to the results and execution of the survey. A firm decision regarding the comparative superiority of any one test can not be justifiably formed. Nor would the opinion that all tests have equal merit be warranted. It is considered that the scope of this survey was too limited to permit valid conclusions. Sampling inadequacies were conspicuous. There were too few total sera and too few sera in each clinical group. Categorical conclusions derived by the other serologists involved may be questioned because, of all the sera included in the survey, significant numbers were either lacking or were insufficient in quantity to undergo testing by all participating laboratories. The number and the types of sera examined by all participants therefore differed, giving no common basis for comparison. To circumvent these inequalities only results with sera (205 out of 248) tested by all laboratories are presented (Table II). Under these better-balanced conditions the differences observed between tests are inconsequential. Conversely, the opinion that all tests have equal value is superficial in view of the quantitative limitations.

The survey has been prepared for presentation not because it accomplished its purpose, but because it may be of general or historical interest.

Comparison of the Sensitivities of Cardiolipin, Kahn and Kolmer-Wassermann Antigens - "Sensitivity" as used herein describes the magnitude of an antigen's reaction with antibodies of reactive sera from presumably syphilitic patients.

During the year cardiolipin antigens became available and the sero-diagnostic possibilities were investigated. It was incorporated experimentally into routine and special procedures and in surveys occupied a prominent off-stage role.

Late in September cardiolipin microflocculation and complement fixation antigens were received from the AMDR&GS. During the month of October the relative sensitivities of these and the routine Kahn and Kolmer-Wassermann antigens were determined by examining in parallel sera submitted to the 406th.

2663 specimens were studied but only 638 arrived in sufficient quantity to permit examination by all four qualitative tests. (538 of these were Kahn-reactive and 115 were Kahn non-reactive sera). (Table III). (201 reactive and 1809 non-reactive sera which had been examined by at least one but not by all four tests were excluded).

Using some of the same samples (total of 276) simultaneous Quantitative Kahn and Quantitative Cardiolipin Flocculation Tests were performed. For purposes of this presentation the end point of the Kahn Test was arbitrarily used as a standard for comparison with the Cardiolipin results. In four instances the Cardiolipin end-point was two tubes lower than the Kahn, in 38 instances there was only one tube difference or the results were identical, in 166 instances the Cardiolipin end-point was two tubes higher, and in 68 instances the Cardiolipin end-point was three tubes higher.

This study also afforded substantiating evidence that the Kolmer-Wassermann Test is satisfactory as a confirmatory procedure in that it compared favorably with the cardiolipin flocculation test. Its value as a confirmatory test is further enhanced since it is used to check all sera showing the slightest Kahn reaction.

Table II. Sensitivities and Specificities of Complement Fixation Tests for Diagnosis of Syphilis

Clinical Classification	No. of Sera Exam.	Saizawa			Browning			Test Kitasato			Sachs			Kolmer-Wassermann		
		Neg	Dou	Pos	Neg	Dou	Pos	Neg	Dou	Pos	Neg	Dou	Pos	Neg	Dou	Pos
Normal	99	96	0	3	90	5	4	93	2	4	93	2	4	96	0	3
Undiagnosed Hos. Patient	10	6	0	4	0	2	8	6	0	4	6	0	4	5	0	5
Syphilis																
Primary Treated	5	0	0	5	0	0	5	0	0	5	0	0	5	0	0	5
Secondary Untreated	12	5	0	7	1	1	10	2	0	10	3	0	9	1	0	11
Treated	39	2	0	37	1	0	38	2	0	37	2	1	36	1	0	38
Tertiary Treated	10	4	0	6	3	0	7	3	0	7	3	0	7	3	0	7
Total	66	11	0	55	5	1	60	7	0	59	8	1	57	5	0	61
Percent		17		83	7	2	91	11		89	12	2	86	8		92
Malaria	3	3	0	0	3	0	0	3	0	0	3	0	0	3	0	0
Tuberculosis	20	19	0	1	18	1	1	19	0	1	19	0	1	19	0	1
Leprosy	6	5	0	1	5	0	1	5	0	1	5	0	1	5	0	1
Pleurisy	1	1	0	0	1	0	0	1	0	0	1	0	0	1	0	0
Total	30	28	0	2	27	1	2	28	0	2	28	0	2	28	0	2
Percent		93		7	90	3	7	93		7	93		7	93		7
Grand Total	205															

Table III. Comparison of Standard Kahn, Cardiolipin Microflocculation, Kolmer-Wassermann and Cardiolipin Wassermann Qualitative Tests.

Tests	Positive No.	Positive %	Doubtful No.	Doubtful %	Negative No.	Negative %
Table I	115 Non-Reactive Sera /					
All four	0	0	0	0	115	100
Table II	538 Reactive Sera x					
Kahn	258	48.0	184	34.2	96	17.8
CL. Floc.	494	91.8	18	3.4	26	4.8
K-W	448	83.2	20	3.7	70	13.1
CL.W	428	79.5	40	7.4	70	13.1

/ Absence of Kahn Reaction

x Kahn reaction ranging from very slight to 4/

Adoption of Supplementary Technics - If reactions with the Quantitative Kahn Test were questionable or indicated additional study, the Quantitative Kolmer-Wassermann Test was put into use as a supplementary procedure. Presumptive and Supplementary Kahn Tests were performed when uncertain results with Standard Kahn Tests suggested their use. Cardiolipin antigens were also used in special cases.

Alsever's Solution - Studies on the preservation of sheep erythrocytes by Alsever's Solution were carried over from the preceding year. After storage for 40 days no changes which might militate against their use in serologic procedures were noted. After 3 months some hemolysis and cell fragility developed. However, when washed, the cells proved satisfactory for routine complement-fixation procedures. Sheep cells preserved in Alsever's Solution were shipped weekly to the three large hospitals in Japan.

Rh$_o$ Antibody Titrations - Recorded technics to detect and quantitate Rh$_o$ antibodies in sera were surveyed. The best features of several tests were combined with methods worked out locally. Early in 1948 a composite procedure was adopted as standard.

This standard test is constantly checked for reliability and validity. Rh$_o$ antibody tests reported in the literature are examined for possible advantages in parallel with the SOP. Of the extra-departmental technics studied thus far none recommended itself for unqualified adoption.

Through December 245 sera from 109 different obstetrical patients were tested. No antibodies were found in the sera of 107 of these patients.

The following points perhaps afford partial explanation for this low figure: (1) About 10% of the specimens are submitted as serum from individuals labelled Rh$_o$-negative. In the absence of accompanying cells it is not possible to confirm or deny the Rh$_o$ factor classification. It has been observed that on occasions certain submitting laboratories err in classifying patients as Rh$_o$-negative; the possibility exists that in some instances the section may be titrating sera from Rh$_o$-positive rather than Rh$_o$-negative patients. (2) It is also possible that requests for antibody titrations are made only for patients in early gestation. It would be advisable to follow these cases through late pregnancy when antibodies are more readily demonstrated. It is recommended therefore, especially when husbands of Rh$_o$-negative patients are Rh$_o$-positive, that the rules for "Rh Testing in Pregnancy" as laid down by circular letter MED 241-1 (W8) Headquarters Eighth Army, Office of the Surgeon, be faithfully followed. (3) It is possible that the proportion of first pregnancies to total number of pregnancies is higher in the selected population contributing samples to this laboratory than is found in an average American city.

FEAF Survey - Sera from 861 airmen selected at random from the Far East Air Forces were Kahn-tested as a special request. 853 were negative, 5 were doubtful and 3 positive.

Research

The Quantitative Complement-Fixation Test - Early in 1948 active work on the unit of 50% hemolysis began. In recent years the trend to estimate complement activity by the end-point of 50% hemolysis has grown noticeably.

Beginning with the observation of Leschly (1) in 1914 and those of Morse (2, 3) and Brooks (4), evidence was presented which pointed out the desirability of titrating complement to the end-point of 50% hemolysis. Wadsworth, Maltaner, and Maltaner (5, 6, 7, 8) systematically applied this technic to diagnostic tests for syphilis, tuberculosis and gonorrhea. In 1943 Friedewald (9), followed in 1946 by Mayer, Eaton, and Heidelberger (10) and by Kent, Bukantz, and Rein (11), independently showed that electro-colorimetric devices enhanced the accuracy and reproducibility with which the unit of complement could be titrated. (The amount of complement producing 50% hemolysis is the unit of complement, hereafter designated K.) It has been established that precision in evaluating soluble hemoglobin spectrophotometrically depends on how faithfully the Lambert-Beer Law is followed. A variety of factors, some beyond control, cause deviations from this Law. Errors due to instrument limitations, instability of the constituents examined and defective operating conditions contribute to the inaccuracies.

These considerations merit attention because spectrophotometry is being increasingly applied to complement-fixation studies. Most clinical laboratories are equipped with instruments which, with respect to hemoglobin analyses, do not invariably fulfill the requirements of the Lambert-Beer Law. It therefore seemed desirable to devise a method designed to minimize errors in spectrophotometric determinations of the hemolytic activity of complement. It is the intent of this in-progress report to

describe the 50% end-point titration with particular reference to (1) selection of the optimal wavelength for measuring hemolysis; (2) utilization of a technic which tends to correct the error caused by deviation from the Lambert-Beer Law and (3) reduction of subjective influences which affect adversely determination of the precise complement unit.

The data to be reported here derived from a combination of several technics. The basic titration scheme and the straight line plot were employed according to the recommendations of Kent (11) and Mayer (10), respectively. Modifications as seemed warranted were introduced independently.

Materials - 1. Diluent (11). All reagents were diluted with 0.85% NaCl made up in 0.005 M phosphate buffer, pH 7.3. The diluent required each day was made up to 1:20 in distilled water from a stock solution containing 170 g of NaCl, 2.78 g of KH_2PO_4 and 11.3 g of Na_2HPO_4 per liter.

2. Erythrocytes. Two hundred ml. of sheep blood were collected aseptically in a 600 ml. transfusion vacuum bottle containing 200 ml. of modified Alsever's solution (12, 13). This mixture was stored at 4° C.

3. Amboceptor. Anti-sheep erythrocyte hemolysin preserved with an equal volume of glycerol was used. A stock 1:100 solution was prepared by diluting a volume of the glycerolized amboceptor with 49 volumes of diluent. The optimal amboceptor dilution required for complement titrations was determined as described by Kent (14).

4. Complement. Lyophilized complement, reconstituted as designated by the manufacturer, was diluted 1:20 with diluent.

5. Spectrophotometer. A Coleman Junior Clinical Spectrophotometer, model 6, was employed to determine degrees of hemolysis. Standard Kahn tubes of 10 mm inside diameter were selected by calibration with a given solution of hemolyzed sheep cells. Only those tubes yielding an optical density of 0.400 ± 0.01 at 550mu wavelength were used.

Selection of Optimal Wavelength - Concentrations of hemoglobin are most accurately measured at a wavelength compatible with the requirements of the Lambert-Beer Law. When spectral-optical density curves were plotted the flat portions of the curves (at which the demands of the Lambert-Beer Law will be met) were found at 500 and 550 mu. Furthermore, these wavelengths will promote the most sensitive response to the different concentrations of hemoglobin examined. Conversely, at 450, 580 and 600 mu, situated on the sides of the curves, the Lambert-Beer Law may not be expected to hold and sensitivity of measurement will diminish.

To verify these observations an arbitrary 100% hemoglobin concentration was prepared by lysing a 2% suspension of sheep cells in four volumes of distilled water. Lesser hemoglobin concentrations were obtained by diluting the 100% solution accordingly. These different concentrations were expressed as "actual percentages". Optical densities for each determined at various wavelengths are shown in Fig. 1.

The curves represented by plots at 450, 580 and 600 mu deviate from a straight line. Those for 500 and 550 mu, while closer, also depart from linearity. With reference to the curve for 600 mu the relative smallness of the deviation is merely apparent. On inspection it will be noted that this diminution is due to the reduced optical density range at which values for 600 mu must be read. When optical densities are converted into calculated percentages (calculated percentage is 100 times the quotient of the optical density for a known concentration ("actual percentage") of hemoglobin and that for the 100% hemoglobin concentration), a base line common to all wavelengths is obtained, thus permitting realistic comparison between deviations at the different wavelengths.

If the Lambert-Beer Law is followed, the calculated and actual percentages will correspond and their plotted points will fall on a straight line (common base line). Deviations from the Law occur and the plotted points form curves as shown in Fig. 2.

The curves plainly show that deviations are greatest at 450, 600 and 580 mu, least at 500 and 550 mu. Their magnitude is maximal around the 50% point.

Percentages of hemoglobin were also calculated using the optical density at 50% hemoglobin concentration instead of that at 100%. (These calculated percentages were obtained by dividing optical densities of known concentration of hemoglobin by twice the optical density for 50% hemoglobin, then multiplying by 100.) These calculated values plotted against corresponding actual percentages gave the curves shown in Fig. 3.

OPTICAL DENSITY-HEMOGLOBIN CONCENTRATION CURVES AT DIFFERENT WAVELENGTHS

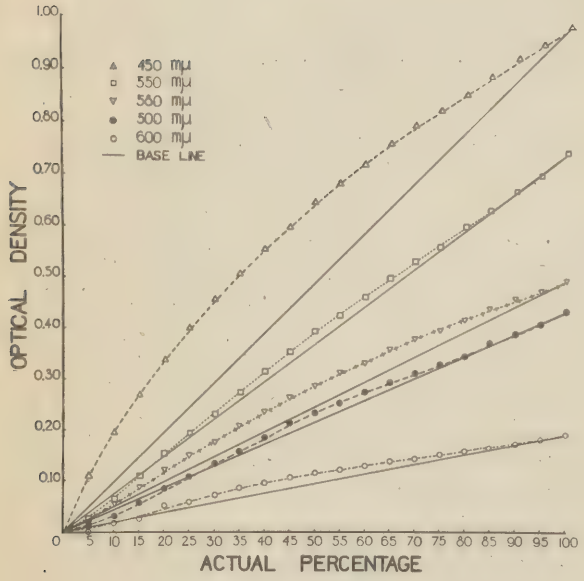


FIG. 1

RELATIONSHIP AT DIFFERENT WAVELENGTHS BETWEEN ACTUAL AND CALCULATED PERCENTAGES

$$\text{CALCULATED \% HEMOLYSIS} = \frac{\text{OD OF KNOWN (ACTUAL) Hb CONCENTRATION}}{\text{OD OF 100\% Hb CONCENTRATION}} \times 100$$

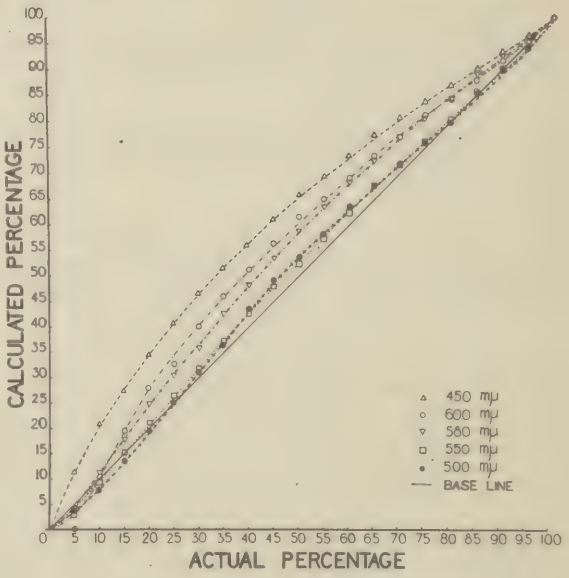


FIG. 2

RELATIONSHIP AT DIFFERENT WAVELENGTHS BETWEEN ACTUAL AND CALCULATED PERCENTAGES

$$\text{CALCULATED \% HEMOLYSIS} = \frac{\text{OD OF KNOWN (ACTUAL) Hb CONCENTRATION}}{2 \text{ OD OF 50\% Hb CONCENTRATION}} \times 100$$

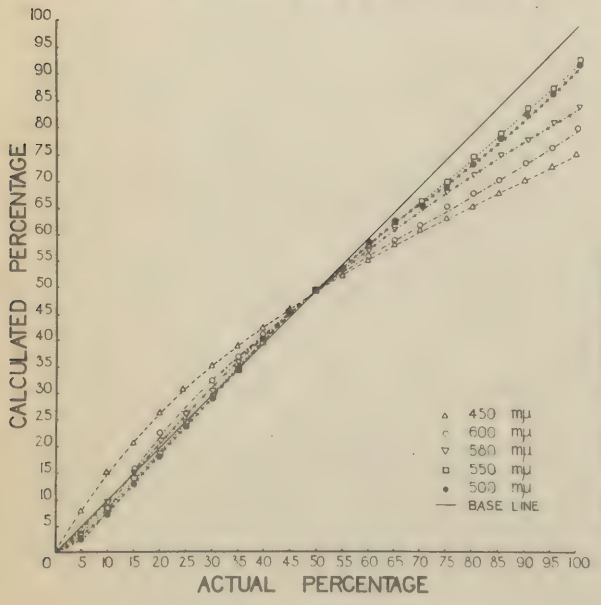


FIG. 3

DETERMINATION OF K:

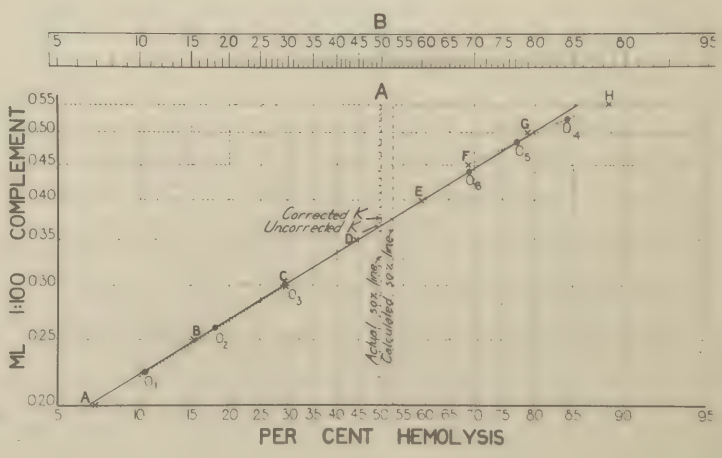


FIG. 4

Again, if the calculated and actual values correspond, their plotted points will fall on a straight line (common base line), thus satisfying the requirements of the Lambert-Beer Law. However, it will be observed that all curves deviate from the base line but intersect it at the 50% and 0 points, where the calculated and actual percentages coincide. Again, departures from the base line are minimal for 500 and 550 mu.

The data summarized suggest that strict linear relationship between optical density and hemoglobin concentration was not obtained at any wavelength used. However, at 500 or 550 mu, conditions for fulfillment of the Lambert-Beer Law seemed best. Because little difference in merit between the two was observed, a wavelength of 550 mu was arbitrarily chosen for routine complement titrations. This wavelength is in reasonably close agreement with that (545 mu) recommended by the Department of the Army for estimating hemoglobin in blood.

Standardization and Sensitization of Sheep Erythrocytes - A suitable quantity of blood preserved with modified Alsever's solution was removed aseptically and centrifuged. The sedimented cells were washed three times with diluent and made up from a packed state to about 2.5% suspension. Two-tenths ml of this cell suspension was added to 1.8 ml of distilled water. The resulting hemoglobin solution was centrifuged and the optical density (OD) of the supernate determined at 550 mu using a distilled water reference. From the reading so obtained the additional volume of diluent needed to adjust the cell suspension to an optical density of 0.400 $\pm$ 0.01 was calculated as follows:

$$\text{Volume to be added} = V \frac{OD - 0.400}{0.400}$$

where V = quantity of sheep cell suspension to be adjusted.

From the adjusted suspension a hemoglobin solution was prepared as indicated above and the optical density of its supernate taken. If a value of 0.400 $\pm$ 0.01 was not obtained, further adjustment was made. The standardized preparation corresponds to a 2% suspension having a concentration of 500,000 erythrocytes per mm^3.

A volume of this cell suspension was mixed with an equal volume of the optimal dilution of amboceptor and the mixture poured back and forth ten times. The sensitized cells were kept at room temperature for at least 20 minutes before use.

Titration of Complement - 1. General Procedure - The method of Kent (11) was adopted with the following modifications: (1) Two sets of hemolysis references were employed, a 100% standard and a 50% standard. (The 50% standard represents hemolysis of one-half the quantity of sensitized cells used for the 100% reference and titration tubes). (2) For the inactivated 1/100 dilution of complement, the zero reference contained all reagents used in the titration set. A sample protocol is presented (table IV).

2. Determination of the Unit of 50% Hemolysis (K) - After incubation and centrifugation optical densities of the supernates of all tubes were determined at a selected wavelength using the blank as reference zero. When a delay in making readings was anticipated, the supernates were poured off and refrigerated.

Two methods served to establish the exact K value. Both involved the use of a logarithmic plot as recommended by Von Krogh (15).

Method 1. The mean optical density of the three 100% hemolysis reference tubes was obtained. The percentages of hemolysis of the other tubes, the 50% reference set as well as the titration set, were calculated by applying the relation

$$\text{(I)} \qquad \text{Calculated \% hemolysis} = \frac{OD}{OD_{H_{100}}} \times 100$$

where OD_{H100} indicates the mean optical density of the reference set for 100% hemolysis and OD represents the optical density of each of the other tubes.

The percent of hemolysis for each titration tube was plotted against its corresponding volume of 1:100 complement on a standard graph form (Fig. 4), the construction of which was suggested by earlier workers, conspicuously Morse (3) and Thompson and Maltaner (15).

Table IV. Sample Protocol for Titration of Complement

	TITRATION					REFERENCE H_{100}			REFERENCE H_{50}			BLANK H_0
TUBE NUMBER	3	4	5	6	7	9	10	11	12	13	14	15
Complement, 1:20, ml						0.30	0.30	0.30	0.30	0.30	0.30	
Complement, 1:100, inactivated, ml												0.30
Complement, 1:100, ml	0.30	0.35	0.40	0.45	0.50							
Diluent, ml	0.90	0.85	0.80	0.75	0.70	0.90	0.90	0.90	1.30	1.30	1.30	0.90
Sensitized cells, ml	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.40	0.40	0.40	0.80
Incubate in water bath at 37 C for 30 minutes. Centrifuge at 1500 rpm for 10 minutes												
Optical density at 550 mu	0.211	0.319	0.421	0.490	0.563	0.713	0.713	0.712	0.376	0.376	0.375	
Mean optical density							0.713			0.376		
Calculated % of hemolysis Method I	29.6	44.8	59.1	68.8	79.0					52.7		
Method II	28.1	42.5	56.1	65.2	75.0							

°To simplify the presentation Tube 1 (which contains 0.20 ml of complement, 1.00 ml of diluent, and 0.80 ml of sensitized cells), Tube 2 (which contains 0.25 ml of complement, 0.95 ml of diluent, and 0.80 ml of sensitized cells), and Tube 8 (which contains 0.55 ml of complement, 0.65 ml of diluent, and 0.80 ml of s ensitized cells) are not shown.

xInactivated at 56 ° C. for 30 minutes.

This form is constructed by applying Von Krogh's alternation equation (16):

(1) $$x = K\left[\frac{y}{1-y}\right]^{1/n},$$

or in logarithmic form,

(2) $$\log x = \log K + \frac{1}{n}\log\left[\frac{y}{1-y}\right]$$

In these relations x represents the volume of complement used and y the corresponding percentage of hemolysis. A plot of log x against $\log \frac{y}{1-y}$ results in a straight line. The constants, 1/n and K, respectively, denote the slope of the hemolytic curve and the amount of complement giving 50% hemolysis (one unit).

The ordinate values shown in the standard graph form indicate the amounts of complement routinely used in titrations. They are spaced logarithmically and can be set off by employing a slide rule. The abscissa values represent the logarithmus of $\frac{y}{1-y}$.

$$\text{For } y = 50\%,\ \log \frac{y}{1-y} = \log \frac{0.50}{1-0.50} = \log 1 = 0$$

$$\text{Similarly, for } y = 30\%,\ \log \frac{0.30}{0.70} = \log 0.429 = 0.367$$

For any unit of length selected, a point on the abscissa 0.37 units to the left of the 0 (50%) point is set off and marked 30%. Other percentages are spaced accordingly with negative values assigned to the left and positive values assigned to the right of the midpoint (50%).

Alternately, the A or B scale of a slide rule can be employed to set off the points on the abscissa. A horizontal line is drawn and an origin representing 50% ($y = 0.50$) assigned. The distance on the slide rule from 5 to 95 is set off left of the origin and marked 5%. The distance from 10 to 90 is set off and marked 10%. The other percentage points are similarly designated until the origin is reached. Percentages greater than 50 are set off to the right of the origin in a like manner.

To interpolate points intermediate to those indicated on the graph, a rule is calibrated in increments of one throughout the range of 5 to 95% (Fig. 5B).

To eliminate subjectivity in fitting a straight line to the plotted points the following procedure was adopted: Using a straight edge the midpoint, O_1, between A and B was marked; the midpoint, O_2, between O_1 and C was marked, and similarly other midpoints were set off progressively until that coordinate on or just left of the 50% line was reached. In like manner midpoints from H to E were progressively determined. Using O_3 and O_6 as guide points a straight line was drawn through them. It is believed that this method reduces subjective factors in fitting a line and also gives appropriate weight to values closest to the 50% point.

If the Lambert-Beer Law is faithfully followed, calculated and actual percentages should be identical. Hence, the ordinate of the point at which the plotted line intersects the 50% abscissa line should represent K. In practice, however, such was not the case. Invariable the calculated and actual 50% values differed. This finding conformed with the observations presented in Fig. 2. It is evident from the curves that deviations occurred at all points of hemolysis but were maximal at the 50% zone. Since 50% hemolysis is the criterion for estimating the unit of complement, it became rapidly apparent that a corrective procedure was necessary to determine the exact K value.

Correction was achieved by projecting a perpendicular from the calculated value of the 50% reference set (52.7% for the conditions shown in Fig. 4). By referring to the ordinate of the point at which this line intersected the plotted line, the exact unit of complement was determined.

Method 2. The mean optical density of the 50% reference set was obtained. Percentages of hemolysis of the titration set were calculated from the relation

$$\text{II} \qquad \text{Calculated \% hemolysis} = \frac{OD}{2OD_{H_{50}}} \times 100$$

where $OD_{H_{50}}$ is the mean optical density of the 50% reference set, and OD represents the optical density of each tube in the titration set.

The different amount of complement used in the titration and their calculated percents of hemolysis were plotted as in method 1; a straight line was fitted to the coordinates.

The ordinate of the intersection of the plotted and 50% abscissa lines designates the exact quantity of complement required for 50% hemolysis (K). Apparently, use of equation II enabled the calculated value of the 50% reference to coincide with the actual value. Hence, further correction was not necessary.

3. K Values Uncorrected and Corrected as Determined at Various Wavelengths - Routine complement titrations were carried out. Degree of hemolysis was measured at different wavelengths then converted into percentages and plotted according to methods 1 and 2. The quantities of complement required for 50% hemolysis were read off the straight line graphs and tabulated.

Table V. K Values[x] Obtained at Different Wavelengths by Method I and II

TITRATION		WAVELENGTH, mu 450	500	550	580	600	MAXIMAL VARIATION
		ml of complement diluted 1:100					
Method I	Uncorrected	0.325	0.374	0.371	0.351	0.345	0.049
	Corrected	0.377	0.379	0.379	0.379	0.381	0.004
Method II		0.382	0.379	0.380	0.382	0.384	0.005

[x] The tabulated figures represent averages of a series of titrations.

The results of several titrations are summarized in Table V. These data indicate that (1) Uncorrected values of K varied widely with the different wavelengths employed; (2) The correction procedures of methods 1 and 2 brought about close agreement regardless of the wavelengths used; (3) Difference between uncorrected and corrected values were greatest at 450, 600 and 580 mu, and smallest at 500 and 550 mu. Since results with 500 and 550 mu correspond so well, a wavelength of 550 mu was arbitrarily chosen for all spectrophotometric analyses; (4) Method 1 seemed to provide slightly better agreement for all wavelengths used than did Method 2.

Practical Application - During this period attention was directed to practical application of the 50% technic. As a starting point the methods practiced by the New York State Department of Health were followed faithfully. Although the New York State technics may be satisfactory in that institution, for smaller, less-specialized laboratories substantial difficulties are imposed. The test is complicated and demands a highly-trained staff. Definitive analysis of each serum requires three separate tests, considerable quantities of reagents and an abundance of glassware. Furthermore, the procedure demands more time than most laboratories can expend on routine serum specimens. Applied to large-scale and survey needs the New York State 50% quantitative technic becomes ponderous.

By promoting greater precision and sensitivity, the present technic enhanced quantitation of antibody levels in luetic sera. However, the pattern of serum dilutions showed relative complexity and, like the New York State procedure, militated against large-scale use.

When initiated here, the primary intent was to study the applicability of this precise technic for such purposes as evaluating vaccine response, antibody response in experimental animals, etc. Initial studies suggest that the technic may be most valuable for this purpose, and that relatively minor changes may be measurable. For example, the titer of a rickettsial antiserum was found to be 1:80 when determined by the criterion of complete hemolysis, while that obtained by the 50% method was 1:350. From experience with the typhus-antityphus system it appears that antigens are most satisfactory when they are soluble or reasonably pure and free from complement-altering properties.

Before proceeding intensively with application of the 50% technic to diagnostic tests, it was decided to inquire further into the reagents of the indicator system. Diluents, complement, amboceptor and erythrocytes were studied with regard to the kinetics of hemolysis. Factors of time, temperature, pH and concentration received primary attention. Attempts were also made to develop homogeneous red cell populations, to stabilize the activity of complement and to devise a simple method for determining amboceptor optima.

Plans For 1949

Cardiolipin - During the coming year it is planned to incorporate cardiolipin antigens into routine examinations of syphilitic sera. Should ample stocks of these antigen become available, they will be used for special projects and surveys as well.

Studies - Improved Rh_o factor and Rh_o antibody tests will be sought. Data resulting from antibody titrations will continue to be accumulated in an attempt to correlate incidence of sensitization with pregnancy.

It is hoped that reagents of the Rh complex in addition to the Rh_o antiserum can be procured so that more definitive analyses can be made.

Japanese Surveys - It is anticipated that there will be additional participation in Japanese surveys. These projects will attempt to select the best flocculation and complement fixation tests for the sero-diagnosis of syphilis.

Evaluation of Kahn Tests Performed by U. S. Army Laboratories in FEC - The Kahn Test as carried out by U. S. Army laboratories in the Far East Command will be surveyed. Samples of sera will be distributed to all participants with directions that the procedure outlined in TM 8-227 be rigidly followed.

Unit of 50% Hemolysis - Investigations of basic mechanisms operating in the indicator system will continue. Alterations and improvements in titrating complement and amboceptor will receive further attention.

Complement fixation tests for the diagnosis of viral and rickettsial diseases will be utilized. Along experimental lines complement-fixation tests will be applied to special problems currently studied in different sections of the 406th.

CHEMISTRY SECTION

Routine

The section functions as a Chemical Laboratory serving the Far East Command. A breakdown of the work performed is given in Table I.

Table I. Routine Chemistry Examinations

Type	Specimens		Tests	
	Subtotal	Total	Subtotal	Total
Clinical Chemistry		1799		2937
Blood	1465			
Feces	140			
Spinal Fluid	137			
Urine	72			
General Chemistry		1751		9512
Blood Alcohol	1027		963	
Toxicology	95		5520	
Water	129		1828	
Whiskey	74		700	
Miscellaneous	426		501	
Totals		3550		12449

Clinical Chemistries - One of the major functions of the Chemistry Section is the analysis of clinical specimens (blood, urine, spinal fluid and feces) submitted by hospital units, whose laboratories are not equipped to perform all the less common analyses. Samples are very frequently submitted from nearby hospitals. Considerable numbers of blood alcohols are requested by authorities in the metropolitan area of Tokyo.

Methods employed in this part of the laboratory are procedures adapted wholly or in part from the TM 8-227. Rapid assays, whose reliability can be justified by published reports, are preferred to those which are time-consuming and whose accuracy is not sufficiently greater to warrant being employed. A set of Standard Operational Procedures has been prepared but is not permanent because the section is constantly evaluating the methods in the light of new information and published reports.

During the year there was a steady increase in the number of tests required by the section. No seasonal trend has been noticed in any of the tests performed.

Toxicology - Toxicological analyses have been performed on selected autopsy tissues submitted from Japan, Korea and the Marianas-Bonin Islands. The largest number of requests have been made by the Pathology Section of this unit. In most of the cases adequate histories were submitted to clarify the chemical approach to the examination.

Specimens for examination were brought to the section in a rather steady stream. There was no way of predicting the amount of work and regularly each month samples were submitted. Due to the highly specialized nature of this work, practical experience in the isolation and identification of poisons is very restricted. Consequently, this area of the laboratory has required a great deal of attention. The attitude was fostered that the physical performance of the Section's tasks, and all its assays dealt with matters which might come before law courts. The workers in this section were fully trained chemists, with long experience in analysis, and some medical background.

A brief review of the positive findings may be of interest:

Ethyl Alcohol - Although conclusions should be left to those who have more data to evaluate, it cannot fail to be significant that one-third of all autopsy subjects submitted to the Chemistry Section showed excessive concentration of alcohol in their bodies at time of death.

Cyanide Poisonings - 1. S.L. -- cyanide was found in brain, liver and stomach contents. History showed a sudden death. Cyanides were detectable four days after death.

2. ES. -- cyanide was found in stomach contents only, but not in brain or liver.

Chlorides - Five cases were submitted in which drowning was suspected. In two there was more than 100 mgs % difference between the right and left heart samples, in two there was no significant difference, and there was one border-line case.

Methyl Alcohol - 1. C.P. -- Brain tissue had 1.4 mg/ml, liver 1.5 mg/ml and blood 0.1 mg/ml. The subject was suspected of drinking methyl alcohol prior to his death.

2. R.J. -- Brain tissue had 7.8 mg/ml and liver, 9.7 mg/ml. It was established by analysis that the liquid which was imbibed by the subject contained a high concentration of methyl alcohol.

Cresols - 1. C. -- The subject was alleged to have been observed drinking lysol. All organs contained large amounts of meta and para cresols.

2. J.M. -- Only traces were found, and no testimony supported suicide by cresols.

Chloral Hydrate - 1. T.T. -- This case was substantiated as due to chloral hydrate poisoning.

2. J.W. -- This case indicated the probable presence of chloral hydrate.

Water Analysis - Samples which arrived at the laboratory have frequently been accompanied by requests for the determination of certain specific constituents. Occasionally no definite requests were forthcoming, and it was left to the decision of the section to choose what tests should be made. In the beginning of the year the number of water specimens which were being received monthly were few. Two technicians, one working only part time, were engaged in water testing. As large numbers of samples were received, considerably more attention had to be paid to this phase of the Section's work. The actual techniques employed (large adapted from "Standard Methods of Water Analysis", A.P.H.A.) were critically examined to see whether tests could be carried out in series. Some of the assays did lend themselves to this (e.g. Silica, Fe and Al, Ca and Mg), and a system of analysis was selected for use in the laboratory and it is successful at the present time. In addition all of the Water Analyses were examined and compared to those employed by various authorities (e.g. New York State Laboratory Methods (1946), the Official and Tentative Methods of the Association of Official Agricultural Chemists, 6th Edition, "Scott's Standard Methods of Chemical Analysis", 5th Edition, and Ryan's "Water Treatment and Purification", 1948), and the Section's standard procedures were adjusted to make the most economical analysis of the sample submitted and to supply values for the information of qualified agencies.

Table II. Routine Chemical Tests On Water

	For Potability	For Boiler Use
Turbidity in ppm	X	X
Color in ppm	X	
Odor, hot and cold	X	
Taste, hot and cold	X	
pH, electrometric	X	X
Sediment	X	X
Residue, total, suspended, dissolved, fixed	X	X
Alkalinity or acidity	X	X
Ammonia Nitrogen	X	X
Nitrite Nitrogen	X	
Total Hardness		X
Chloride	X	X
Sulfate	X	X
Nitrate	X	X
Oxygen Consumption	X	
Silicate	X	X
Iron, Aluminum and Phosphate	X	X
Calcium	X	X
Magnesium	X	X
Manganese	X	X
Sodium and Potassium		X

The Section has been developing the use of standard methods for the quantitative determination of turbidity, and of color, in water in terms of parts per million. Japanese clay has been obtained, and graded to 200 mesh for use as a standard. For color a cobalt platinic-chloride salt is used.

Further, it was felt that greater uniformity in tests performed might be preferable to the engineering section most interested in the water examinations. Consultations were held with various submitting agencies with a view to standardizing the Water Analysis Procedures and securing sufficiently comprehensive data for the interpretations of the interested engineers. Table II presents the routine tests evolved from these conferences.

Food Analysis - Following the general policy of the Far East Command the Section's interest in foodstuffs has been confined first, to those specimens submitted by medical officers who feel that some irregularity in the food has adversely affected the well-being of people in their care, and secondly, to those specimens submitted by Veterinary Corps officers asking for specified chemical tests. Hence, only samples of questionable nature have been tested.

The approach to these problems has been to make chemical analyses to detect the presence of contaminating toxic substances, or for constituents in unusual amount, or for decomposition of the original product. Laboratory procedures are those adopted from toxicological manuals (e.g. Simmons and Gentzkow), or from food analysis manuals (e.g. "Methods of Analysis, A.O.A.C.", 6th. Edition). Wherever specifications have been established, it may suffice to determine whether a food meets these qualifications. If the food has produced certain reactions, it may be possible to test for the presence of specific contaminants.

The following is a brief summary of some of the examinations:

Caviar. This product, a "Romanoff Brand", was submitted by the Post Exchange. It had white particles of crystalline appearance, clearly visible in the glass jar container. The material was apparently denatured protein.

Soya Flour. Eight samples of this flour were submitted for blanket tests. The flour was under suspicion of being the cause of food poisoning. Only one sample was found contaminated, and this was a sample scarped from the floor near the flour sacks.

Oysters. Two one gallon cans of "Sealshipt" brand oysters were submitted by the Yokohama Surgeon because the shipment from which they were sampled had not been completely refrigerated. The oysters were found not to comply with the rigid federal specifications (pH variation).

Ground Beef. The fat content of this Quartermaster hamburger mix was under question. It was found, however, to meet specifications.

Powdered Milk. This sample of "Klim" was found to be uncontaminated, but possessed a stale, unpleasant odor, probably due to protein and fat changes.

Sterilized Milk. The milk was of the "Avo" brand type, and, although suspected of souring, was found to be well preserved.

Tea. This Chinese Tea was thought to be a source of narcotics, but was found to be harmless.

Baking Powder. Two samples, English and Australian, were examined to determine whether they served as vehicles for narcotics. Both were as labeled.

Pepper. This spice contained no contaminant or denaturant as was suspected.

Cocoa. The cocoa, an English name-brand, was quite edible and contained no foreign substances.

Cheese. "Velveeta" brand cheese was found to contain glass-like particles which were proven to be protein-lactose granules. The cheese, although edible and nutritious, was not desirable because of its gritty taste and glass-like appearance.

Skimmed Milk. The milk was submitted to determine its protein, fat and carbohydrate content, since it had been prepared by a hospital laboratory for patient consumption. It was found to be quite good.

Tea. This product was a brewed tea, submitted because it was thought to be poisoned. No toxic materials were found.

Raw Sugar. This Japanese consumer product was alleged to have caused sickness. Since it was one of a series of similar cases, the section was asked to examine it for contaminants. None were found.

Fluid Milk. Fat content of this product prepared for pediatric patients was below standard.

Cheese Containers. New Zealand cheese was supplied in cans whose inside surface was not properly prepared before canning. The cheese was shown to have a large iron contamination as a result of this improper treatment.

Food analysis, by the Section, is expected to continue in much the same manner in the future - as a toxicological problem, not as a routine food check.

Miscellaneous Analyses - This term encompasses the manifold qualitative and quantitative investigations carried out constantly by the Chemistry group. Requests for such analyses flow in from many sources, including various laboratories, prisons, depots, military government teams, Public Health and Welfare Section, Counter Intelligence Corps, Economic and Scientific Section and Military Police Division. In most instances, each analysis poses a separate problem. They are, probably, a real example of the unpredictable work which must be carried out by a Medical General Laboratory and serve as an illustration of the nature of the service which the chemistry laboratory must be able to offer.

Because the work is of a varied character it does not lend itself readily to rigid classification. A loose association has been made, however, to classify the samples received by the laboratory and the following listing is serviceable:

Pharmaceuticals - Sulfa tablets (18), Dextrose-Saline (60), Sodium Sulfadaizine (18), Penicillin (5), Hexylresorcinol, Elixir Terpine Hydrate (2), Maltose, Atropine, Pills (3), Tablets (7), Ointments (2), Hormone, Histine, Sodium Citrate (3), Sodium Chloride (2), Milk of Magnesia (2), Procaine (2), Culture Media (2), Dextrin.

Narcotics: Marihuana (9), Powders (45), Liquids (10), Morphine (3), Opiates (6), Cyanides (2).

Heavy Chemicals: Gases (15), Metal, Tar, Water (4), Explosives (4), DDT (5), Chemical Kits (9), Ice, Formaldehyde (3), Insecticides (11), Methyl Alcohol, Sulfuric Acid, Potassium Permanganate, Ethyl Alcohol (83), Soap (16) Hypochlorites (3), Tallow.

Others: Beer (19), Hydrogen Ion Determination, Whiskey (55), Lactose in Urine, Milk Sediment, Gastric Contents, Boiler Scale (2), Color Comparators (3), Herb, Kidney Stones (2), Permanent Wave Lotion.

Approximately 500 specimens were taken care of in the miscellaneous Analysis group. In no case has a qualitative analysis failed to identify the substance under investigation. It is difficult to make a complete report of the number of tests which were necessary to perform before identities were established just as it would be difficult to list those procedures not done because they were contraindicated. Some of these analyses, with their background deserve brief attention:

Sulfa Tablets: Early in the year, and sporadically during the remaining months, the laboratory analyzed bogus tablets of the various sulfa drugs confiscated by the military authorities, or subjected to question by medical investigation personnel. In general the tablets were well prepared by expert manufacturers. The formula in all cases was the same; a minute amount of any sulfa drug, some starch, and a binder. The sulfa drug used was rarely the one purported to be in the tablet. Sulfanilamide, sulfathiazole and sulfaguanidine were used as token ingredients.

Whiskey and Beer: Samples of liquors are submitted frequently to the section. When they do arrive they are tested for specific contaminants. A high incidence of methyl alcohol, over 10% of all samples, has been found in the whiskeys analyzed, a dangerous percentage even when based only on the suspect liquor which demands analysis.

Medical Department Products: These have frequently been submitted because of alleged reactions. Although the section has not acted to set up specification, it has constantly served to check specifications previously established (such as pharmacopoeia requirements). The section has examined dextrose-sodium chloride solutions, hypochlorite powder, sulfadiazine ampules, sodium citrate anticoagulant, procaine dental anesthesia, ethyl alcohol and many of the other Medical Department Product items. With these, as with the Dextrose-Sodium Chloride Solutions, general procedure was to check the materials to determine whether they met pharmacopoeia requirements, or similar established specifications.

Gas: Analysis of gases requires specialized equipment, not available to the section. Nevertheless, because of the need to control the quality of oxygen for Medical Air Force units, apparatus suitable for assay of gases, such as oxygen or carbon dioxide, has been devised.

Insecticides: The Public Health and Welfare Section, SCAP, has done a great deal to foster the use of DDT in Japan. They have made the material available to Japanese jobbers, and have successfully advocated legislation granting funds for commercial utilization of DDT in powders and sprays. However, Japanese legislators allegedly backed a program for the use of Japanese insecticides. Seven of those insecticides ("Nippol", "Baktol", "Neopop", etc.) were accordingly submitted to the section for assay. Far from being what they were claimed to be, these products were completely unreliable - weak, poorly prepared, lacking in declared ingredients, in most cases inert.

Explosives: Four different samples were submitted for identification as explosives. One was said to be ammonium nitrate, but was merely gravel. Another material, molded in the shape of a shell packing, was found to be powdered iron, salt and asbestos. A third material (from Korea) was actually an explosive - sulfur, charcoal and nitrate. A fourth material (also from Korea) proved to be effective as a fuse, its probable intended purpose.

Special

Determination of Procaine Hydrochloride - The procedure developed for this determination consists essentially in the diazotization of procaine with nitrite, coupling the product with alphanaphthylamine and measuring the intensity of the color produced. The readings are best carried out at a wavelength of 535 mmu.

Estimation of Thiocyanates in Blood - A spectrophotometric method for the determination of thiocyanates in blood was adopted by utilizing the procedure of Barker (1936) (12). The method consists in the formation of the iron thiocyanate color in protein-free blood filtrate, and measuring the intensity of the color in the spectrophotometer at a wavelength of 550 mmu. The method does not account for all the thiocyanate in the blood and it appears that 100 percent recovery of the thiocyanate present does not occur.

Adoption of a Blood Salicylate Assay - Salicylate assay in the blood was requested several times during the year. The method of Keller (1947) (13) was adopted by this laboratory after being checked.

Determination of Total Protein - A micro-Kjeldahl procedure was adopted for the determination of total protein. This method is very accurate but is time-consuming, and is utilized only for special purposes.

Sodium and Potassium Assays - These are time-consuming and generally unsatisfactory. Although it appears that the most reliable assay of sodium should employ a flame-photometer - which is expensive- studies were begun to find a reliable, inexpensive method.

Animal Colony - A breeding colony of mice was started to provide suitable animals of known age and weight for controlled studies.

Research

Barbiturate Analysis - Because of the dangerous popularity of the use of barbiturates for sedation, a need was felt for a quantitative method for the determination of barbiturates in tissues. A search of available literature (1, 2, 3, 4) revealed the existence of several qualitative and semi-quantitative methods. The reaction of barbiturates with the cobaltous ion in an anhydrous alkaline medium appeared a most likely method for intensive investigation, particularly since it was felt that it could be adapted to the spectrophotometer.

This work has not yielded clear-cut results and many difficulties have been encountered. Koppanyi (5) reported successful use of a method similar to the one being developed in this investigation. His work is being investigated at the present time, and it has been demonstrated that the reaction as described in the literature (5, 6) is subject to some criticism: there is variation of color intensity with time; and suitable reference blanks are not clearly described. Present findings in this laboratory have not yet been evaluated. A synopsis of the work performed is presented here to indicate what has been established at this point.

Reagents - 1. Alkaline Medium: several alkalis were tried; sodium ethylate, Japanese triethanolamine, barium oxide, barium hydroxide, pyridine and aniline. None of these were practical. Isopropylamine was found to be a suitable product for this purpose.

2. Cobaltous Ion: dehydrated cobaltous nitrate, and dehydrated cobaltous acetate were prepared by heating the hydrated salts at various temperatures ranging from 100° C to 800° C. The products obtained were either insoluble in alcohols, or partially decomposed. Cobaltous nitrate ($6H_2O$) was adopted as the most practical reagent since it was soluble in alcohol in sufficient quantities to yield significant color with barbiturates.

3. Solvent: ethyl alconol, methyl alcohol and chloroform were tested as suitable solvents for the reaction medium. Ethyl alcohol was selected as the most practical.

Spectral Transmittance Curve - This curve was obtained with the above reagents and showed a minimum at 565 mmu.

Optimal Concentrations of Reagents - 1. Concentrations of 0.2 mg barbital and less cannot be assayed.

2. In 12 ml solution, 250 mg of isopropylamine and 1.5 mgs of hydrated cobalt nitrate may be sufficient to determine 0.5--3.5 mgs. of barbital.

3. In 12 ml solution, 100 - 150 mg of isopropylamine and 5.0 mgs. of hydrated cobalt nitrate may be sufficient to determine 2.0 - 4.5 mgs of barbital, and possibly more.

Barbiturate Intoxication and its Relation to Alcoholic Consumption and Impaired Liver Function - An investigation was initiated to ascertain the effect of barbiturate poisoning, when the organism is affected by alcoholic consumption, or by liver damage due to a high fat diet, or by liver damage due to poisons. This work was prompted by a desire to investigate whether or not any relationship existed between these conditions in toxicological findings and to train members of the section in the techniques of small animal dietary studies preparatory to initiating a combined program with another section of the laboratory dealing with the response to highly infectious agents of animals deficient in certain factors.

At the present time, the studies have been confined (a) to a study of the effect in mice of simultaneous administration of ethyl alcohol and sodium barbital, and (b) to a study of the effect of the administration of sodium barbital to mice with nutritionally induced liver impairment. The mice were initially subjected to subcutaneous barbiturate injection to determine the LD_{50} (and LD_{100}) of the poisons. Then the LD_{50} of the barbiturate in mice who were consuming various amounts of alcohol or excessive fat was determined, and this value was compared to the earlier figure. In these studies, the LD_{50} was calculated according to Trevan's (6) interpretation of the median lethal dose as one which kills 50% of the animals in a large series.

The median lethal dose of Sodium Barbital - Medical Supply Item JAN, Cat. No. 1-092-350, Barbital Sodium, $\frac{1}{4}$ lb., was used. One hundred fifty-five normal six week old German strain mice were injected subcutaneously with 0.1 ml. of an aqueous solution of sodium barbital. Each ml. contained either 120 or 128 mg. of barbiturate, and the amount of sodium barbital varied from 0.50 to 1.04 mg. per gram body weight. It was ascertained that the LD_{50} of sodium barbital for six week old mice lies between 0.70 and 0.74 mg per gram body weight and that the LD_{100} is approximately 0.95 mg per gram body weight. At any given dose, the average number of hours for death to occur is always less than the average number of hours required for recovery.

The median lethal dose of Sodium Pentobarbital - The sodium pentobarbital used for subcutaneous injection was extracted in pure form from JAN Item No. 1-330-770, Pentobarbital Sodium Capsules. On the basis of a group of 70 mice it appears that the LD_{100} for mice is 0.23 mg of sodium pentobarbital per gram body weight. The LD_{50} lies somewhere between 0.18 and 0.20 mg/g. This was a preliminary study and has not been further pursued since it is planned to confine the present study to one barbiturate.

The median lethal dose of Sodium Barbital in mice administered Ethyl Alcohol - Four week old normal German strain mice were obtained and kept for an additional two weeks on water and dehydrated food (7.a.) At the end of this time selected groups of the mice were given an alcoholic solution to drink instead of water. One hundred ml of the alcoholic solutions contained either 2 or 4 ml of absolute alcohol, 2 grams of dextrose, and water. Mice were fed ad lib. dehydrated food and given the alcoholic solution as their only supply of fluids. A careful record was maintained of the amounts of water consumed by the control group and of the amounts of alcoholic solution consumed by the test groups. No difference in the total average volume of fluid ingested was noted, the average per day per mouse averaging 4.5 ml, with a range of 3.4 ml to 5.6 ml.

Complete records were maintained in each of the studies to be presented, and have been subjected to statistical treatment. The various methods of analysis are similar for each group and to avoid repetition detailed treatment has been presented for only one group (those ingesting 4% alcoholic solution).

1. The effect of Sodium Barbital injected subcutaneously on mice fed 2% alcohol in water.- One hundred thirty-nine normal six week old German strain mice were given a 2% sugared solution of ethyl alcohol to drink in place of water for two weeks. A control group consisting of one hundred thirty mice were given plain water as their liquid for the same period of time. At the end of the two week period the alcoholic and control mice were injected with 0.10 or 0.15 ml of sodium barbital solution. The total dose given to each mouse was either 11.1 or 16.6 mg.

In this group the LD_{50} for sodium barbital seems to lie between 0.60 and 0.64 mg per gram body weight for both the control and the test animals. Both groups gained weight equally well, averaging 4.2 gms per mouse during the two week period (range 3.5 - 5.1). There was no difference in the average time required for anaesthesia to become complete, nor were the extreme ranges of time required for complete anaesthesia significantly different. No difference could be found in the response of males and females.

2. The effect of Sodium Barbital injected subcutaneously on mice fed 4% alcohol in water - One hundred eighteen normal six week old German strain mice were given a sugared 4% solution of ethyl alcohol to drink in place of water for two weeks. A control group consisting of one hundred fifteen mice were given water for the same period of time. At the end of the two week period the alcoholic and control mice were injected with 0.10 or 0.15 ml of sodium barbital solution. The total dose of barbiturate given to each mouse was either 11.1 or 16.6 mg.

Table III shows the effect on eight week old mice of subcutaneous injection of the sodium barbital solution. (At the age of six weeks the mice had been placed on the dehydrated food diet and (A) or (B) liquid.)

Table III. Toxic Effect of Sodium Barbital on Mice Fed 4% Ethyl Alcohol

Av. Dose[x] mg/g	No. mice injected	No. mice dead	Dead (Av. hours)	Recovered (Av. hours)
	(A) 4% sugared alcoholic solution			
0.52	4	0	--	15
0.57	6	1	28	22
0.62	18	6	25	26
0.67	16	9	35	30
0.72	12	9	30	31
0.77	12	10	21	33
0.82	12	9	22	28
0.87	15	14	17	24
0.92	11	11	9	--
0.97	7	7	7	--
1.02	5	5	5	--
	(B) Water			
0.52	6	1	42	15
0.57	11	7	40	25
0.62	11	8	40	31
0.67	14	10	40	19
0.72	12	11	22	30
0.77	10	10	17	--
0.82	4	3	25	48
0.87	19	17	12	25
0.92	19	17	8	26
0.97	5	5	6	--
1.02	4	4	6	--

[x] Av. Dose: 0.52 represents 0.50-0.54, 0.57 represents 0.55-0.59, etc.

Trevan's method for calculating the LD_{50} (median lethal dose) consists of combining any two consecutive ranges of doses and calculating the mortality, i.e.:

1. Alcoholic mice

0.60-0.69; 54 injected, 15 dead; 44%
0.65-0.74; 28 injected, 18 dead; 54%

2. Control mice

0.50-0.59; 17 injected, 8 dead; 47%
0.55-0.64; 22 injected, 15 dead; 68%

Although more data is necessary to substantiate this difference, it appears that the LD_{50} for 4% alconolic mice is 0.65-0.69 (by Reed-Muench formula 0.67) in contrast to 0.55-0.59 (by Reed-Muench formula 0.59) mg per gram body weight for the control group. The LD_{100} for both control and alcoholic groups is approximately the same, being in the vicinity of 0.95 mg of sodium barbital per gram body weight.

Table IV which follows lists the average number of minutes required for alcoholic and control mice to become completely anesthetized (comatose, with the complete absence of convulsions) when a specific volume and quantity, 0.10 ml (11.1 mg) or 0.15 ml (16.6 mg), of sodium barbital was injected subcutaneously.

Table IV. Time Required to Completely Anesthetize Mice with Sodium Barbital

		Completely Anesthetized (Minutes)			
		(A) 0.10 ml (11.1 mg)		(B) 0.15 ml (16.6 mg)	
Av. Dose[x] mg/g	Effect	Alcoholic	Control	Alcoholic	Control
0.55	Death	36	39		
	Recovery	38	45		
0.65	Death	31	32	35	29
	Recovery	39	34	29	--
0.75	Death	24	23	24	27
	Recovery	33	33	29	--
0.85	Death	18	15	26	20
	Recovery	--	--	32	21
0.95	Death	18	18	23	26
	Recovery	--	--	--	19
1.05	Death	15	19	19	16
	Recovery	--	--	--	--

[x] Av. Dose: 0.55 represents 0.50-0.59, 0.65 represents 0.60-0.69, etc.

The expected trend seems to be present here: as the dosage is increased, the time for complete anesthesia of the mouse is decreased.

As was done with all the group, the weight of every mouse in both alcoholic and control groups was recorded at the start of the two week experimental feeding period and again on the day of the injection of sodium barbital.

Table V which follows gives the average weight of the mouse at six and eight weeks. The average gain in weight is also tabulated.

Table V. Studies on Weight Gain of Mice

	0.10 ml.		0.15 ml.	
	59 Control Mice	66 Alconol Mice	57 Control Mice	61 Alcohol Mice
Av. weignt at six weeks (grams)	12.3	12.3	14.8	15.8
Av. weignt at eight weeks (grams)	16.8	16.2	19.2	19.0
Av. gain in weight in two weeks (grams)	4.5	3.9	4.4	3.2

Table VI. Toxic Effect of Sodium Barbital on Male and Female Mice Fed 4% Ethyl Alcohol

Av. Dose[x] mg/g	No. mice injected		No. mice dead		No. mice injected		No. mice dead	
	Alcoholic Mice				Control Mice			
	M	F	M	F	M	F	M	F
0.52	2	2	-	-	4	2	1	-
0.57	1	5	-	1	3	8	1	6
0.62	5	13	2	4	7	4	5	3
0.67	6	10	3	6	2	12	1	9
0.72	3	9	2	7	7	5	7	4
0.77	6	6	4	6	7	3	7	3
0.82	7	5	5	4	2	2	1	2
0.87	9	6	9	5	9	10	9	8
0.92	5	6	5	6	9	10	7	10
0.97	2	5	2	5	1	4	1	4
1.02	3	2	3	2	3	1	3	1

[x] Av. Dose: 0.52 represents 0.50-0.54, 0.57 represents 0.55-0.59, etc.

It appears that both the control and alcoholic male and female mice follow the same pattern of mortality.

3. The effect of Sodium Barbital in Mice Fed a 20% Fat Diet - Studies are in progress but an insufficient number of animals have been observed to permit deductions.

Investigation of Cadmium Sulfate as an Agent for Measuring Liver Disease - In 1947 Wunderley and Wuhrmann (8) reported a cadmium flocculation Method which apparently served to measure increases in blood serum protein resulting from liver damage. This method was extremely simple, used common reagents and required only 0.1 ml of serum. Because of difficulty in securing cephalin cholesterol antigen, there was a need for a reliable test which would not demand unusual reagents. In addition the authors were employing a visual subjective, plus-rating of the turbidity produced by the cadmium-protein flocculation, which appeared worthwhile adapting to the spectrophotometer. It was, therefore, considered advisable to investigate the possibilities presented by the Wunderley-Wuhrmann assay.

The approach to the assay was two-fold. A relationship between the flocculation produced by a cadmium salt in sera, and the clinical background of patients whose sera is employed is being sought. At the same time the results of the cadmium sulfate test are being compared with those obtained by two other accepted liver function tests, viz. the thymol turbidity test (McLagan, 1944) (9), and the cephalin cholesterol flocculation test (Hanger, 1939) (10).

The accuracy of the thymol turbidity test depends on the visual acuity of the technician and on the standard turbidity solution prepared for visual subjective comparison: it hinges on the turbidity produced by thymol-globulin aggregations, which is then compared to a series of barium sulfate standards (Kingsbury, 1927) (11). It was decided that a spectrophotometric adaptation of the thymol turbidity test was necessary for purposes of comparison.

Special transmittance curves were plotted for the thymol turbidity reaction and the cadmium salt (sulfate) reaction. Sera were employed which produced high turbidity in both cases. A minimum graph reading was obtained with the cadmium reaction at a wavelength of 420 mmu. This wavelength was established with different sera. No minimum value was obtained with the thymol reaction. The arbitrary value of 650 mmu was selected because this value had been used by other authors.

Optical Density reading of thymol turbidities was carried out in the following manner:

1. Tube 1. 0.1 ml serum and 6 ml buffered thymol solution.

2. Tube 2. 0.1 ml serum and 6 ml saline (blank).

3. These are compared directly

a. With Kingsbury's barium sulfate standards.

b. By optical density measurement, at 650 mmu, using buffered thymol as a reference. The turbidity value is equal to the difference in the optical density of Tubes 1 and 2. (R_1-R_2).

c. By optical density measurement at 650 mmu using Tube 2 as a reference for Tube 1. (R_3).

It was found that a turbidity value could be obtained by measuring the optical density of a thymol serum sample against a saline-serum blank, thus eliminating a buffered thymol blank as reference. This can be shown by the following calculation on 77 sera:

$R_1 - R_2$ = Turbidity value using buffered thymol as a reference.

R_3 = Turbidity value using saline serum as a reference.

$\frac{(R_1 - R_2) - R_3}{R_3} \times 100$ = % Difference in Result.

The values obtained are listed in the following tables:

% Difference	0.0 - 1.0	1.1 - 5.0	5.1 - 10.0	11
% Sera Showing the Difference	40.2	52.0	5.2	2.6

Typical examples of those turbidity values showing a % difference of about 5% are shown in the following Table:

Serum	R_1	R_2	R_1-R_2	R_3	% Difference
A	0.047	0.002	0.045	0.043	- 4.7
B	0.193	0.014	0.179	0.188	- 4.8
C	0.024	0.001	0.023	0.022	+ 4.6

A comparison was made of the Optical Density readings of the thymol reaction and the subjective visual comparison of the thymol reaction with barium sulfate standards. There was a general agreement in results by the two methods.

Comparing the cadmium sulfate test to the thymol turbidity test and cephalin cholesterol flocculation test introduces certain pitfalls. It was shown that these tests may not give related values. The following table demonstrates this quite well (normal thymol reading is 1--4, normal cephalin reading is 0--1 plus).

Thymol Turbidity Values

Cephalin-Cholesterol Readings	1--4	5--9	10--20	over 20	Total
Neg. or 1 plus	24	18	7	2	51
2 plus	5	2	5	1	13
3 plus	8	14	3	2	27
4 plus	7	14	5	6	32

It was also shown that no correlation exists between the thymol turbidity assay and the cadmium sulfate assay. These results are charted in Figure I. The cadmium sulfate test was carried out as follows:

1. Tube 1. 0.6 ml serum, and 0.3 ml 0.4% $CdSO_4$.

2. Tube 2. 0.6 ml serum, and 0.3 ml saline.

3. These are measured by optical density readings at 420 mmu, using cadmium sulfate solution as a reference. The turbidity value is equal to the difference in the optical density of tubes 1 and 2. Readings were made on 77 sera.

The effect of stability of sera on the cadmium sulfate test was examined by maintaining sera for varying periods at room temperature and under refrigeration. An obvious drop was noted in the cadmium sulfate readings in all instances. It appears that this drop is more apparent in specimens under refrigeration.

Plans for 1949

In the forthcoming year present barbiturate studies will be continued. It is expected that the data obtained will be reasonably complete and adequate for conclusions to be drawn on the following projects:

a) Alcohol-barbiturate synergism.

b) Barbiturate assay.

c) Cadmium liver function test.

An investigation will be made to determine the effect of a diet, nutritionally inadequate in thiamine, riboflavin, pyridoxine and pantothenic acid on the production in animals of antibodies to Japanese B encephalitis virus. By using synthetic diets, deficiencies will be produced in mice and hamsters. These animals will then be inoculated with the virus, and their antibody formation or other reaction will be observed.

As part of the regular Clinical Chemistry work, the section will continue to investigate methods of analysis for substances which are requested in sufficient numbers to warrant setting up a standard operational procedure. One of these investigations is a suitable method for the assay of 17-ketosteroids in urine, another is the determination of lead in blood, a third, the assay of sodium in blood.

In Water Analysis, this section will work toward setting up a uniform series of tests for each type of water specimen submitted so that waters everywhere in Japan can be compared.

BACTERIOLOGY SECTION

The following sub-units comprise the Section of Bacteriology: Diagnostic Medical Bacteriology, Bacteriology of Water and Foods, Coprological Studies, T. B. Diagnosis, Biological Assay, Biologics Production, Media, the Animal Room and Central Supply.

Routine

During the calendar year 25,379 specimens were processed in the routine diagnostic unit. This number included 2,050 routine diagnostic specimens, 17,778 water samples, 559 ice samples, 1,948 milk, cream and ice cream specimens and 2,544 routine bacterial agglutinations.

Approximately 2,000 stool specimens were submitted for bacteriological study. Suspect tuberculosis specimens numbered 1,037. Data on these last two categories is expanded under various later sections of this report.

Biologics - For the period of this report 126 bacterial strains comprising 807 units were lyophilized and 193 additional types were maintained pending lyophilization. Four hundred and twenty 5 cc. units of antigen, 160 units (5cc.) of antisera, fifty-two 0.3 cc. units of thromboplastin and 50 units of prescribed autogenous vaccines were prepared.

The following is a catalogue of non-AMDR&GS cultures maintained in this laboratory for purposes of reference and study:

(a) Escherichia coli (97-C-K), (98-A-K), (122-A-2), (130-A-1).

(b) Streptococcus pyogenes (C-203-M), (C-203-S), (B-225), (C-440), (C-441), (NY-5), (H-46-A), (Blackmore), (CH-39).

(c) Lactobacillus casei (Snell).

(d) Vibrio comma (Inaba F-29), (Inaba F-30), (Inaba F-31), (Inaba F-32), (Inaba F-33), (Inaba F-34), (Ogawa T-10), (Ogawa T-11), (Ogawa T-12), (Ogawa T-13), (Ogawa T-14), (Ogawa T-15), (No. 73), (No. 74), (Ogawa TU-1), (Denecke TU-2), (Finkler Prior TU-3), (El Tor TU-4), TU-5), (Hikojima TU-6), (Yanagiwara TU-7).

(e) C. diphtheriae (S77), (M084), (M086), (S173), (g347), (g368), - gravis (Tg-1) to Tg-16), - mitis (Tm-1) to Tm-56), (Km-1), (Km-2), - intermedius (Ki-1), - minimus (Tm-1).

(f) C. ovis (CS1R1), (CS1R2), (CS1R3), (CS1R4), (CS1R5), (CS1R6).

(g) C. ulcerans (170), (37142), (39164).

(h) C. pyogenes (Bullock-14-1(S)), (Bullock-14-1(L)), (Abortion-14-2), (Kidney-14-6), (C-47-14-8), Burrows-14-9), (P14-S), (P14-L), (S-26), (C-7), (C-11), (C-18).

(i) C. hemolyticum (637-S), (637-L), (13081-S), (13081-L), (13853), (14223), (14248), (14275), (14340), (14635), 14636), (15520), (15544), (504-2-S), (Czech No. 1), (Czech No. 2), (Czech No. 3), Czech No. 4), (J1), (J8), (H1), (16172), (16229), (53-W-1), (53-W-2), (502-12D).

Media, Animal Room and Central Supply - 2,521 liters of bulk media, 37,750 plates, 41,219 slants and 47,803 tubes of media were prepared during this year. 301 rabbits, 2,841 guinea pigs and 1,840 mice were employed for the completion of routine, investigative and manufacturing procedures effected during this same period.

Lots of S.S. Agar, Difco, bearing control numbers 364991a, 354765 (5), 364916 and 351939b, have been tested here under carefully controlled conditions and have been found to successfully inhibit the growth of representative strains of Salmonella and Shigella.

The factor or factors in fresh eggs associated with successful recovery of M. tuberculosis, apparently are greatly diminished by short periods of aging. Day old eggs as required for Corper's Medium

and Jensen's Modification of Lowenstein's Medium cannot be obtained locally in a continuous supply.

Special

Diagnostic method for tuberculosis - During the calendar year 1,037 specimens were received for study in this division. At this time 114 guinea pig confirmed positives have been reported along with 598 similarly confirmed negatives; 272 incomplete specimens remain in process. The division at present averages 120 specimens per 30 day period.

The routine method for handling specimens for demonstration of M. tuberculosis in this laboratory was outlined in the 1947 report. Briefly it consists of a carefully controlled acid digest of the sample, inoculation of Corper's media (1) neutralization, and of the liquid media of Dubos (2), and inoculation of two guinea pigs. These pigs are tuberculin tested at 4, 6 and 8 weeks following inoculation, and are sacrificed for histological examination on the basis of positive tuberculin reactions. The demonstration of acid-fast organisms in a tissue section with definite lesions compatible with tuberculosis is the final criterion for a positive diagnosis.

1. Changes in the S.O.P. - (a) The use of Tuberculin P.P.D. (Seibert) Second Strength, used originally out of necessity and later for purposes of comparison with O.T., has been discontinued.

Table I

	4 weeks	6 weeks	8 weeks	Total
O. T. Positive	94	23	6	123
P. P. D. Positive	71	19	8	98

In addition to the 123 positives reported in Table 1, two animals negative to O.T., were demonstrated to have lesions of tuberculosis by histological examination. These were the only two failures in dilutions of 1/10, while 11 "failures" were noted at dilutions of 1/25. It would thus appear that O.T. in dilutions of 1/10 as a prognostic reagent is satisfactory. As has been mentioned in previous reports, of 6 non-pathogenic acid-fast bacilli recovered in 422 cultures for Mycobacterium tuberculosis none stimulated in guinea pigs positive reactions to 1/10 O.T. (b) The rapid acid-fast technique recommended by Moran (3) and utilizing Tergitol (Carbide and Carbon Chemicals Corporation, Indianapolis, Indiana) has been given a trial period here with seemingly satisfactory results. (c) The addition of sodium penicillin to Dubos' liquid medium and to Dubos' solid medium produces a selective action useful in the recovery of M. tuberculosis.

Table II

	Inoculum	Growth in Dubos' with Penicillin 5 u/ml.	Growth in Dubos' with Penicillin 10 u/ml.	Growth in Dubos' without Penicillin
1.	M. tuberculosis	(+++)[x]	(+++)	(+++)
2.	Mixture of M. tuberculosis Staph. aureus	M.t. (+++) St. aureus (-)	M.t. (+++) St. aureus (-)	M.t. (+++) St. aureus (+++)
3.	Mixture of M. tuberculosis B. subtilis	M.t. (+++) B. sub. (-)	M.t. (+++) B. sub. (-)	M.t. (+++) B. sub. (+++)

[x] Indicates good growth

In the first 100 digests (sputa, urine and gastric lavage, and one stool) M. tuberculosis was recovered seventeen times on plain Dubos' medium and an equal number of times (actually from identical cases) from Dubos' medium with 5 units of sodium penicillin per ml. added. However, using the plain medium some type of bacterial growth was noted in 46 instances while the addition of penicillin decreased this type of finding to only 19 instances. (d) During the summer months digestion of specimens submitted for T.B. diagnosis had to be carried out at temperatures as much as 15° above those optimal for such operations. It is felt that some recoveries failed perforce of this situation.

2. Accumulated data regarding the value of Dubos' liquid medium in the routine diagnosis of M. tuberculosis - A year ago this Section reported its experience with Dubos' medium up to that time. This medium has been observed as a part of a system employed in the examination of 1,480 digests from which 325 positive results were obtained over a period of sixteen months. In the same system the egg medium of Corper was also employed. Time. Of 141 positive Corper's medium cultures, the mean recovery time was 35 days. (The efficacy of Corper's medium depends much upon the freshness of the eggs. Since this is an uncontrollable factor in the theatre, the comparison here is not fair in terms of Corper's formula, which distinctly states that fresh eggs must be used). The mean time for 246 positive Dubos' cultures was far less (see Table III).

Table III

246 Positive Cultures in Dubos' Medium

⊀ in 5 days	⊀ in 7 days	⊀ in 7 days	⊀ in 14 days	⊀ in 21 days
72	30		51	93

3. Recovery Index - Common problems in the evaluation of the use of any culture medium with unknown exudates are the matter of, first, "positiveness" versus "negativeness" of exudates and, secondly, random distribution in them of the to-be-recovered organism. Random distribution is further complicated by the total number of organisms, their viability, etc. For the data to follow here, the first problem, "positiveness" versus "negativeness" of exudate is solved through the admission to the total specimens to be evaluated, only those specimens which produced tuberculosis in prior tuberculin negative guinea pigs. Such tuberculosis in most cases was secondary to the inoculation of the exudate; in some cases it was secondary to the introduction into guinea pigs of exudate positive cultures. Random distribution of tubercle bacilli in the inoculum was accommodated within certain limits; the entire specimen was cultured and/or inoculated into guinea pigs. The probability that Corper's medium would support the growth of most strains of M. tuberculosis was attested to through long experience with the medium in this laboratory as well as by the experience of its author (1).

Table IV-a

	Corper's (⊀)	Corper's (-)	Total
Dubos' (⊀)	42	27	69
Dubos' (-)	9	22	31
Pigs (⊀)	51	49	100[x]

[x] Total is composed of 89 pigs positive from initial digest and 11 pigs positive secondary to inoculation of positive cultures.

Table IV-b

	G.P. (⊀)	G.P. (-)	Total
Dubos' (⊀)	69	8	69
Corper's (⊀)	49	6	49
	69	II	69

In Tables IV-a and IV-b are summarized work on exudates from one hundred proven cases of tuberculosis selected by choosing one hundred consecutive positive reports from the Pathology Section, this Laboratory, on guinea pigs inoculated from an equal number of humans suspected as possible cases of tuberculosis; 89 of these exudates were proven by initial guinea pig inoculations from the digested specimens; 11 by guinea pig inoculation from cultures positive when the initial pigs were negative.

Since all sputa were, therefore, shown by one way or another to contain pathogenic bacilli capable of producing tuberculosis in guinea pigs, it may be assumed that the initial animal discrepancy of 11% was due to insufficient bacilli or improper distribution of bacilli in the exudate. If Dubos' medium be also considered in the light of this distribution error, i.e. given a 69/89 recovery index, Dubos' medium in the series presented here yields an overall recovery potential of 76% as opposed to initial guinea pig recovery of 89%. When it is considered that all digests processed under the system in use here are partitioned four way for inoculation into Corper's, Dubos' and two pigs, the importance of the corrected figure, 76%, in an evaluation review may be more easily appreciated.

4. Recovery of Saprophytic acid-fast bacilli - Summarized in Table V are figures on the distribution of non-tuberculogenic acid-fast bacilli encountered on smears, in Corper's and in Dubos' medium in the examination of 1,042 specimens submitted for routine laboratory diagnosis for M. tuberculosis.

Table V

Non-tuberculogenic A.F.B.
encountered in the routine processing of 1,042 specimens

Smears	Dubos'	Corper's
8	13	10

Total: 18 (1.8%)

As a source of confusion in the preliminary presumptive reporting on specimens cultured for M. tuberculosis, the incidence of these non-tuberculogenic acid-fast bacilli seems unimportant. Some authors have insisted that experienced workers could easily distinguish between the "saprophytic" mycobacteria and M. tuberculosis on solid media whereas they could not in liquid media. The differential criteria employed by such workers are mode of growth and chromogenicity. In liquid media not containing indicator or other dyes, the chromogenic strains may be detected; some of the non-chromogenic strains may be ruled out on the basis of cell morphology; some not. Certainly there is no cultural means of ruling out such organisms as Calmette - Guerin's bacillus, a non-tuberculogenic strain which bears the taxonomic designation of M. tuberculosis.

5. Summary - There have been 3 previous reports from this laboratory on the use of Dubos' medium in hastening preliminary presumptive laboratory diagnosis of tuberculosis. The data in the first reports simply served to confirm the work of Foley (4) which established the fact that Dubos' medium could be successfully incorporated in methods for the laboratory diagnosis of tuberculosis. The present series of one hundred cases was compiled from routine specimens. Since the majority of such material is submitted on a "rule out" basis, cultural recovery from positives in such a group should be more difficult than in those lots sampled earlier. That this is true seems to be borne out by the figures presented in Table IV-a where sixty-nine cultures were positive as opposed to 89 positive pigs, all out of a proven possible 100 pigs and/or cultures.

The medium of Dubos' is easily prepared and is not subject to broad changes in effectiveness due to uncontrollable variations in its ingredients. Since multiple culture methods with solid media have remarkably increased the number of positive yields, especially where the viable bacilli in the inoculum were scant, the quality, unique to liquid media, of supplying infinite accommodation for bacterial surfaces in a minimum volume gains in importance. This evaluation of Dubos' medium after 16 months work with it may be summed up as follows:

(1) Shortens the time for preliminary presumptive reporting in the absence of a positive smear.

(2) Is easily prepared.

(3) Offers the largest available surface per cc. of any medium currently recommended for the laboratory diagnosis of tuberculosis.

Diagnostic Methods in Gonorrhea - The value of chocolate agar with yeast extract added for a "holding" medium for the transportation of specimens to be cultured for N. gonorrhea has been ascertained in comparison to several other media. The following represents a 60 day progress report.

(1) Media - The media employed in this investigation included two control media, A and B, and three test media, No. 1, No. 2, and No. 3. The control media were dispensed in petri dishes; the test media in 8 oz. screw cap prescription bottles (4-060-310) in such a manner as to yield a lengthwise of-the-bottle sheet of agar 1/4 inch in depth. The ingredients of the various media are summarized in Table VI.

Table VI. Media Employed

Ingredients	Medium				
	Control		Test		
	A	B	I	2	3
Proteose Peptone No. 3 Difco	X	X	X	X	X
Haemoglobin Difco	X	X	X	X	
Yeast Extract 1%	X	X	X		
Human Blood 7.5%	X		X		X
Inactivated Human Serum 10%			X		

Table VII. Recovery of N. gonorrhea on Various "Holding" Media

Percentage Recovery Based on:	Medium					
	Direct			Delayed		
	No. 1	No. 2	No. 3	No. 1	No. 2	No. 3
Clinical Diagnosis (100 cases)	49	51	46	35	44	29
Control Media (58 cases)	84	88	79	60	75	50
Overall Recovery All Media (86 cases)	57	59	53	40	51	33

(2) Exudate - The 100 specimens used were secured with sterile swabs from the cleansed cervices of women interned as cases of clinical gonorrhea at Yoshiwara Hospital, Tokyo.

(3) Method - Swabs containing cervical exudate immediately were streaked to control Medium A, control medium B and to two each of the 3 bottled test media. In an effort not to favor any one medium, random inoculation was aimed at. The inoculated media were next separated into two groups on the basis of the time lapse they were to undergo prior to their incubation. One group, consisting of the two control plates, A and B, and one each of the bottled media No. 1, No. 2 and No. 3, was termed as receiving the direct method: the time lapse between seeding and beginning of incubation for this lot was limited to one hour. The other group, termed as handled by the delayed method, experienced a 24 hour delay at 20°-24°C. before being incubated. Incubation for both groups was carried but under reduced oxygen tension (10% CO_2) at 37°C. for 24 hours. Following incubation, suspicious colonies were picked and their identity established through subsequent fermentation tests. The oxydase reaction was not employed. Colonies were picked at 24 hours but not at 48 hours in the above study. Undoubtedly, the total recovery would have been greater had a second picking been accomplished.

(4) Results - The results of work on 100 clinical cases are to be found in Table VII. On control media A and B only 58 recoveries were made as against 86 recoveries obtained when positive cultures were totaled from the entire 800 units of media used. The discrepancy between these two figures undoubtedly may be attributed in large part to random distribution of the N. gonorrhoeae in the initial inocula. Difference in growth requirements obviously does not appreciably affect this differential of 58-86 = 28% From Table VII it may be seen that with the direct method, incubation of media within one hour following inoculation, Test Medium No. 2 (Plain Chocolate Agar, Difco) yielded a total number of recoveries superior to any of the other test media. With the delayed method, 24 hour delay at 20° -24° C. prior to incubation, the medium giving superior results was still Test Medium No. 2 (Chocolate Agar Difco). With delay, the recoveries were lower, 44 as opposed to 51.

From the foregoing data, the importance of using large surfaces of medium for each specimen cultured seems indicated (overall recovery of 86 as opposed to 58 on the control plates alone). Still to be obtained is information regarding the extent to which test medium No. 2 can be expected to support the growth of fastidious strains of N. gonorrhoeae. It is felt that one of the superior attributes of Chocolate Agar, Difco, in the small series of cultures reported here, was its failure to provide too lush a growth of concomitant organisms.

Bioassay - During the year, 257 titrations for presence of antistreptolysin, 30 assays for penicillin potency, 2 assays for streptomycin potency, 33 tests for penicillin sensitivity, 24 tests for streptomycin sensitivity, 19 tests for sulfadiazine sensitivity, 7 tests for antibiotic activity of unknown substances, 4 tests for oleic acid sensitivity of corynebacteria, 1 test for antihemolytic activity against Corynebacterium pyogenes toxin and 1 test for potency of typhoid vaccine of army were carried out by this division.

The need for exact methods for assaying antistreptolysin in serum from patients suffering and or convalescing from streptococcal infections has been met through the application of the techniques outlined below.

1. Preparation of lysins - Three lysins are available for differential titration of sera submitted to this laboratory: (a) Oxygen labile O-lysin (5); (b) the Oxygen stable S-lysin (6) and (c) the lesser known streptococcal lysin of Okamoto (7) which Bernheimer has designated as S'-lysin. Methods for producing these lysins follow:

A. Preparation of Streptolysin-O.

Strain: Streptococcus pyogenes C-203-M or H-46-A

Medium:

Bacto-beef (dehydrated powder)	50 g.
Proteose-Peptone (Difco)	20 g.
NaCl	2 g.
Na_2HPO_4	1 g.
Dextrose	2 g.
Distilled water	1000 ml.

Infuse Bacto-beef powder in 1000 ml. distilled water at a temperature of 50°C. for 1 hour. Then heat to 80°C. and hold at that temperature for several minutes. Filter. Add peptone, NaCl, Na_2HPO_4 and dextrose, heating gently to dissolve. Adjust pH to 7.6. Refilter. Dispense as required. Autoclave at 15 pounds for 15 minutes.

To the above base, aseptically add maltose solution to a final concentration of M/200 and sodium bicarbonate solution to a final concentration of 0.25%.

The original inoculum should consist of a large volume of rapidly growing organisms taken from liquid culture. The inoculated sub-culture is incubated at 37°C. for 18 hours, then, centrifuged at 2500 r.p.m. for 10 minutes and, finally, rendered sterile by filtration. The filtrate is tested for sterility and, if sterile, is ready for further purification. (Note: While both C-203-M and H-46-A produce both S- and O-lysin, the amount of S-lysin produced under the conditions set forth here has been negligible. The use of Wilson 868, Hodder 872 or Tucker 873, all straight O-lysin producers, as inocula for routine O-lysin production has been contraindicated because of the low hemolytic unit yield).

Concentration of O-lysin - The first part of this procedure is essentially that of Rantz (8). The culture filtrate is brought to 0.57 saturation by the addition of 429 grams per liter of ammonium sulfate at 20°C. The resulting precipitate is removed in the regular centrifuge and dissolved in a phosphate buffer of pH 7.0. The final volume should be about 4% of that of the original culture. This viscous brown liquid is transferred to cellophane sacs and dialyzed for 18 hours against running tap water, then against distilled water for 5 hours.

Nine grams of NaCl and six grams of Na_2HPO_4 are added to each liter of the dialyzed material. The resulting pH should be approximately 7.0. The material is next sterilized by passage through a Seitz filter. After aseptic transfer to sterile tubes, the product is ready for use.

Further purification with about a three fold increase in combining capacity and a five fold increase in hemolytic power may be effected by ethanol precipitation using the method of Bernheimer (9). Lysin material is precipitated with two volumes of absolute ethanol at pH 7.0. The precipitate is sedimented by centrifugation, after which the ethanol is removed. The precipitate is then redisolved in phosphate buffer at pH 7.0 and reprecipitated by the addition of two volumes of absolute ethanol and, finally, dried in vacuo.

B. Preparation of S-lysin.

Strain: Streptococcus pyogenes (Blackmore).

Medium:

Bacto-beef (dehydrated powder)	50 g.
Proteose peptone (Difco)	20 g.
NaCl	2 g.
Na_2HPO_4	1 g.
Distilled water	1000 ml.

adjust pH to 8.0 and autoclave 15 lbs. for 15 minutes.

Supplements:

(1) Maltose Solution M/200
(2) Serum (horse) 20%
(3) $NaHCO_3$ 0.25%

Completed medium requires the aseptic addition of supplements (1), (2) and (3) in the above indicated concentrations. The inoculum consists of the cells harvested from an 18 hour old blood plate previously heavily seeded with S. pyogenes (Blackmore). This harvest is introduced into 50 cc. of the above medium and incubated at 37° for 6 hours. The filtrate obtained from 6 hours growth is the final S-lysin. The lysin deteriorates so rapidly that it will lose all of its hemolytic power after 24 hours storage at 4°C.

C. Preparation of Streptolysin S'.

Strain: Streptococcus pyogenes C-203-M.

Medium:

Bacto-beef (dehydrated powder)	50 g.
Proteose peptone (Difco)	20 g.
NaCl	2 g.
Na_2HPO_4	1 g.
Dextrose	2 g.
Distilled water	1000 ml.

adjust pH to 7.6 and autoclave at 15 lbs. for 15 minutes.

Supplement:

Nucleic Acid (Yeast).

Aseptically add supplement to a final concentration of 1%. Inoculum is prepared as given under S-lysin above. Incubation is carried out at 37°C. for 16-20 hours. The culture filtrate may be used as the final lysin. Further concentration by ethanol precipitation has not been tried.

2. Factors affecting the value of streptolysins as agents used in the assay of immunological response to Streptococcal infections in man. - The three lytic streptococcal metabolites mentioned above are divided primarily on their reaction to atmospheric oxygen. Streptolysin-O is labile to oxygen and reducing agents, such as sulfhydril compounds, are necessary for its activation. It further is antigenic and antisera against it may easily be prepared in rabbits. Both streptolysin-S and streptolysin-S' are stable in the presence of oxygen and are, for all practical purposes, non-antigenic. There has been some question as to whether or not they (S and S') were identical and Bernheimer (10) states that "In all respects studied, however, the nucleic acid hemolysin is identical with Streptolysin S." In this laboratory, however, S and S' have exhibited two apparent differences: (a) pH range for optimal hemolytic activity and (b) degree of stability (see Table VIII).

Table VIII

	ph	M.H.D.	Days stable at 4° C.
S-lysin	8.0	32/cc	<1
	6.8	16/cc	
S'-lysin	8.0	16/cc	10
	6.8	32/cc	

While the difference in titers shown in Table VIII is of an order possibly attributable to pipetting error, it is felt that it represents a difference which, when considered along with the marked evanescence of the S-lysin, is significant. The diagnostic value of differential streptolysin titers is far from understood. Those institutions routinely using antistreptolysin titrations as an experimental part of the laboratory work-up of patients with streptococcal infections are employing, for the most part, only o-lysin. This is probably because of (1) difficulties encountered in working with S-lysin, (2) the over-looking of Okamoto's work (S'-lysin) on the part of Westerners and (3) the non-antigenic nature of S⁻ and S'-lysin. Around the latter point hinges this laboratory's proposed program, should patients with severe streptococcal infections become available, of assaying sera against each of the three lysins. Although it has not been possible to produce antisera against these oxygen stable lysins, both human and rabbit serum exhibit varying abilities to inhibit their lytic action upon erythrocytes. Whether or not the presence of or absence of S⁻ and S'-lysin neutralizing factors in serum may be correlated in any way with the course of human streptococcal infections remains to be seen. It is hoped that a three lysin assay will offer some information on this point.

3. Titration for antistreptolysin or for streptolysin blocking activity of serum - A 5% suspension of less than 4 day old Rabbit R.B.C. is used for all o-lysin determinations; 2% Rabbit R.B.C. suspension is used for S⁻ and S'-lysin.

The titration of serum for anti-O-lysin is adapted from Rantz's method (op. cit.).

A. Determination of combining unit.

(1) Preparation of cysteine solution.

NaCl ..... 4.2 g.
KH_2PO_4 ..... 3.17 g.
$Na_2HPO_4 \cdot 12H_2O$ ..... 3.56 g.
Distilled water ..... 1000 ml.
pH 6.5

To the above solution 1.6 g. of NaOH are added. At the time of testing 0.15 g. of cysteine hydrochloride is dissolved in 25 ml. of this alkaline buffer to make a M/26 solution of the active agent.

(2) Dilution of streptolysin-O.

The streptolysin-O is diluted with isotonic buffer serially in decrements of 2 from 1:2 to 1:16, depending on the hemolytic activity of the streptolysin. Its hemolytic power is determined by adding 0.5 ml. of Rabbit RBC to each tube containing 1.5 ml. of the separate dilutions.

(3) Test:

Samples of each significant dilution are then set up in small tubes according to the schedule in Table II.

Table IX. (Interpolation titration - Combining Unit)

								Control No. 1	Control No. 2
Lysin of given dilution	(ml.)	0.35	0.30	0.25	0.20	0.15	0.10	0.20	----
Buffered cysteine	(ml.)	0.15	0.20	0.25	0.30	0.35	0.40	0.30	----
Standard antistreptolysin	(ml.)	1.00	1.00	1.00	1.00	1.00	1.00	----	----
Saline	(ml.)	----	----	----	----	----	----	1.00	1.50
5% RBC	(ml.)	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50

Reduction is permitted to proceed for 10 minutes when 1 ml. of a dilution of standard antistreptolysin, containing 1 unit per ml. is added to each tube except control tubes. Contents are thoroughly mixed and incubated for 15 minutes in the water-bath at 37° C. Then 0.5 ml. of a 5% suspension of thrice-washed defibrinated Rabbit RBC is added to each tube. (It is desirable to wash and suspend the cells in the isotonic buffer, pH 6.5 without added NaOH). After further incubation for 45 minutes the tubes are removed from the waterbath and centrifuged. That amount of lysin which has just failed to produce hemolysis is the combining unit. This combining unit contained in a volume of 0.5 ml. will be used for the determination of antistreptolysin titer.

B. Determination of anti-O-lysin titer of serum - The antistreptolysin-O of serum is determined by using freshly reduced lysin. The lysin is first diluted with isotonic buffer to the degree determined to be suitable by the previous test. A further dilution in the buffered cysteine solution is then made at the time of use, so that the combining unit will be contained in a final volume of 0.5 ml. For instance, a lysin might be diluted 1:8, 15 ml. of this material being then added to 35 ml. of the reducing solution, 0.5 ml. of which contains the amount of reduced lysin which is completely neutralized by 1 unit of standard antistreptolysin. The lysin is ready for use after 10 minutes exposure to the reducing agent and does not deteriorate for 60 minutes.

Lysin prepared in this way can be used to determine the antistreptolysin titer of each serum in a single operation (by using a simplification of a procedure originally suggested by Hodge and Swift). This can be accomplished by preparing two-fold serum dilutions in isotonic buffer or physiological saline, starting with 1:10. (See Table X).

Table X

Number of tubes	1	2	3	4	5	6	7	8	9	10
Buffer or saline (ml.)	1.8	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.5
Serum (ml.)	0.2	1.0	1.0	1.0	1.0	1.0	1.0	1.0[x]	---	---
Reduced lysin (ml.)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	---
Unit value of each tube	10	20	40	80	160	320	640	1280	---	---

[x] One ml. of this solution is discarded.

After 15 minutes of incubation at 37° C. in a water-bath, 0.5 ml. of the Rabbit RBC are added and the whole reincubated for an additional 45 minutes. The last tube in which lysis has not occurred indicates the anti-O-lysin titer of the serum. A control serum of known antibody titer should be examined in exactly the same way.

Routinely a 10-tube test with two control tubes is carried out, since titers rarely exceed 1:1280. If the titer of a specimen falls between two widely separated dilutions and a more exact endpoint is required, a second titration to encompass the desired range is set up. The order of procedure follows:

(a) Inactivate serum sample at 56° C. for 7 minutes.

(b) Add buffer or saline to series of tubes.

(c) Prepare the proper dilution of lysin and add the proper amount of cysteine solution, to obtain the desired combining unit per 0.5 ml.

(d) Allow 10 minutes for reduction of lysin.

(e) Make serial dilution of serum from 1:10 to 1:1280 using isotonic buffer diluent. Add appropriate volumes of dilution to each tube.

(f) Add 0.5 ml. of reduced lysin to each tube.

(g) Shake well and incubate at 37° C. for 15 minutes.

(h) Add 0.5 ml. of RBC suspension, shake, reincubate for 45 minutes, then read.

The titration of serum for anti-S-lysin - Following inactivation, the serum sample is diluted with borate buffer of pH 8.0. Next a gradient of serum dilutions ranging from 1:10 to 1:320 or higher, if desired, is set up. Then 0.5 ml. of streptolysin-S in borate buffer, pH 8.0 to yield 1:0 M.H.D. is added to each tube. After 15 minutes of incubation at 37° C., each tube receives 0.5 ml. of 2% Rabbit R.B.C. The tubes are then reincubated for 2 hours at 37° C. before final readings are made. For each day's run, S-lysin must be checked for hemolytic endpoint and a buffer control should be set up. A schematic presentation of the foregoing procedure may be found in Table XI.

Table XI

						Control No. 1	Control No. 2
Serum	0.2	1.0	1.0	1.0	1.0[x]	---	---
Borate buffer pH 8.0	1.8	1.0	1.0	1.0	1.0	1.0	1.5
S-lysin	0.5	0.5	0.5	0.5	0.5	0.5	---
R.B.C.	0.5	0.5	0.5	0.5	0.5	0.5	0.5

[x] discarded

If a more exact reading is desired, a secondary titration is set up to accommodate the range indicated by the endpoint obtained in the first titration. For example, if the endpoint for the first titration is in the 1/320 tube, a range from 1/320, 1/340, etc., to 1/640 may be used as the secondary titration.

Titration of serum for anti-S'-lysin - The protocol for setting up S'-lysin titrations is identical with that for S-lysin except that in the case of the former ordinary physiologic saline (pH 6.8) is employed instead of borate buffer, pH 8.0.

4. Discussion - Much work remains to be done before the above techniques can be considered finished methods. A standard M.H.D. for all lysins needs to be employed and should probably be defined as X amount lysin capable of releasing k amount of hemoglobin from c concentration of cells in t amount of time.

Little is known of the combining power of S and S' lysins. Hodge and Swift (11) pointed out that there existed between o-lysin and its homologous antiserum a constant combining ratio over a long period of time in the face of diminishing M.H.D. values. Similar data regarding S and S'-lysins and their specific antisera is not available since both of these lysins are nonantigenic. In an effort to have available one hemolysis blocking agent which might be used for obtaining fixed activity values for all three of the group A streptolysins, an investigation of the possibilities of Congo Red as a suitable blocking substance was initiated. It may readily be weighed and a standard 10 gm. lot could be used

for a long time. Gordon in 1931 demonstrated the hemolysis blocking ability of Congo Red on hemolysins of certain streptococci and clostridia (12). In this laboratory consistent results have been obtained using Congo Red as a blocking agent for S- and S'-lysin. O-lysin requires so much larger quantities of the dye that there is confusion between color resulting from hemolysis and free dye. It is hoped that this difficulty may be obviated by spectrophotometric examination.

It will be noted in the foregoing protocol that 5% Rabbit R.B.C. are employed in O-lysin determinations, whereas 2% Rabbit R.B.C. are used in S and S'-lysin assays. Todd, whose standard anti-streptolysin-O globulin is used in this laboratory, defines an M.H.D. in terms of 5% cells. A 2% R.B.C. suspension is more easily handled, and is used here in tests where it does not interfere with the Todd values.

Diphtherial Toxin Content of Diphtherial Toxoid Used in Immunizing Japanese Children in the Kyoto Area. - Members of this section were requested to aid in the investigation of deaths occurring in certain children in the Kansai District. The following official SCAP news release printed in The Pacific Stars and Stripes, 31 December, 1946, gives general data pertinent to problem investigated.

"According to investigations made by both SCAP and Ministry of Welfare officials, the faulty toxoid was produced by the Osaka Red Cross Research Institute in four five-liter flasks. Even though the contents of the four flasks were not pooled into a common container, the four separate lots of diphtheria vaccine were grouped by the manufacturer, designated as Lot No. 8 and packaged into 1,000 vials of 20 cc each. Judging from the fact that only the vials numbered serially above 500 were at fault, it appears that the third and fourth of the four flasks were not properly detoxified. This was due either to the introduction of insufficient formalin or to insufficient incubation at proper temperatures, or both.

Unfortunately, the eight vials of diphtheria vaccine taken by the Ministry of Welfare inspector and submitted to the National Institute of Health for assay were not representative of the entire lot and failed to show the faulty detoxification step in the manufacturing process."

Important, in addition to the information given in the official release, is the fact that the toxoid remained for almost one year, following bottling, before it was certified for use. All children immunized with Lot No. 8 Toxoid had 5 - 7 days previously received their initial dose of diphtherial toxoid. To the first doses there were no local reactions; to the second (1.0 ml.) there were many.

1. Clinical - Through the generous cooperation of Captain John Glisman, Kyoto Military Government Team, and Dr. Irako, Kyoto Public Health Laboratory, clinical histories of 54 of the 62 children dying following the administration of the second doses of diphtheria toxoid were obtained. Necrosis with ulceration at the site of inoculation was noted in 32 cases, local oedema in 52, urinary failure in 9, axillary lymphadenopathy in 7 and pharyngeal paralysis, as evidenced by difficulty in swallowing, was noted in 34 cases. Results of relatively few urine examinations are available. Of 19 urinalyses albumin was reported 17 times, while casts and red cells were recorded 6 and 7 times. It should be pointed out here, that the clinical records were the work of several different Japanese physicians and that there was marked variation in their general approach. Hence, there may have been a greater frequency of the above mentioned symptoms than was indicated by their data. The causes of death for the 54 cases as noted by attending physicians were as follows: "Heart failure and post diphtheritic paralysis", 18; "pneumonia", 10; "general emaciation", 2; paralysis of the diaphragm and pharyngeal muscles", 17; cellulitis and ulceration of the left upper arm", 1.

2. Assay of the Toxoid - 250 gram guinea pigs were employed as test animals. Forty random sampled ampoules of Lot No. 8 Osaka Red Cross Research Institute toxoid were screened for toxicity through the use of 0.2 cc doses administered intradermally in guinea pigs. Those samples giving rise to marked reactions were further screened using 0.1 cc doses and, finally, toxoid from an ampoule (338) yielding an average severe reaction was selected for MLD determinations.

250 gram pigs of good health were used, four for each dilution. The classic MLD determined at 96 hours, using that amount of toxoid which killed 50% of the test animals, proved to be 0.1 ml. All of the animals were further observed for thirty days in an effort to ascertain lethal doses based upon longer periods of time. These data are the basis for the graph comprising Figure 1. It may be noted that the toxic values for the toxoid ranged from 10 MLD per ml. for the ninety-six hour lethal time to 25 MLd per ml. for the 30 day lethal time. The graph in Figure 2 records the date of death from the time of vaccination for 55 children receiving 1 ml. of toxoid containing the classic 10 MLD (i.e. maximum lethal time is 96 hours) which is the equivalent of 25 MLD, when the maximum lethal time is 720 hours or thirty days. Roughly, the greatest number of children to die died in a period between

DATA CONCERNING DIPHTHERIAL TOXOID USED IN KYOTO

MLD CONTENT OF OSAKA RED CROSS TOXOID WHEN THE LETHAL TIME FOR GUINEA PIGS IS EXTENDED PAST NINETY-SIX HOURS

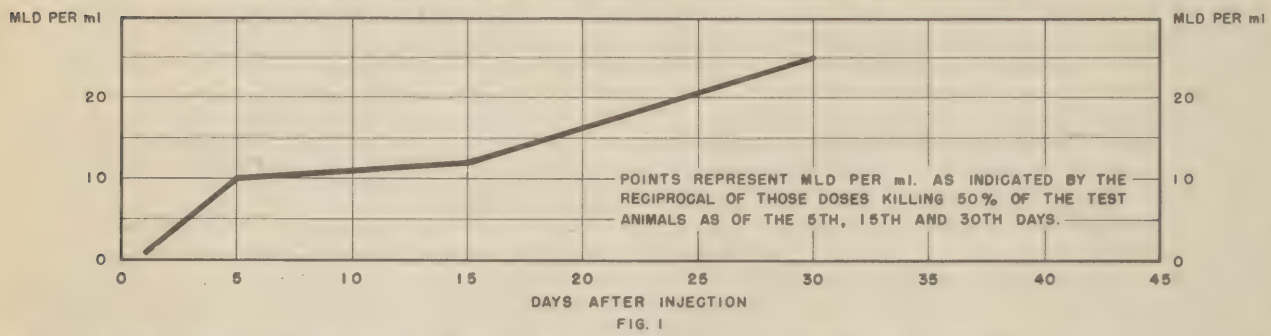


FIG. 1

LETHAL TIME FOR CHILDREN DYING BEFORE THE 45TH DAY FOLLOWING ADMINISTRATION OF 1.0 ml. OF TOXOID NO. 8 PREPARED BY THE OSAKA RED CROSS INSTITUTE FOR MEDICAL RESEARCH

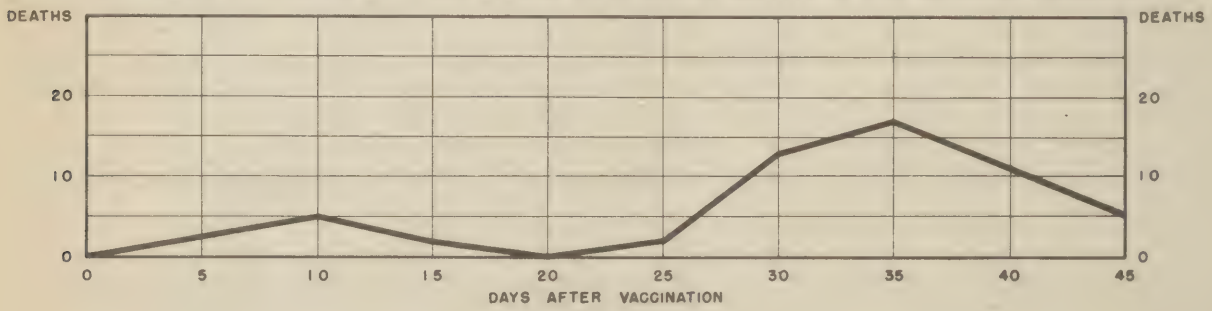


FIG. 2

AGES IN MONTHS IN TERMS OF LENGTH OF ILLNESS TO TIME OF DEATH

DAY OF DEATH FROM DATE OF VACCINATION	10	15	25	30	35	40	45
AVERAGE AGE IN MONTHS	17	41	10	11.5	17.5	13	24.8

TABLE XIV

AVERAGE WEIGHTS IN KILOGRAMS FOR VARIOUS AGES OF JAPANESE CHILDREN OF NORMAL HEALTH*

SEX \ AGES IN MONTHS	10	11.5	13	17	17.5	24.8	41
MALE	8.70	9.05	9.33	9.95	10.03	11.14	13.37
FEMALE	8.21	8.58	8.82	9.33	9.40	10.53	12.81

* QUOTED FROM "IKUJI TO CHIRYŌ YORI MITARU SHŌNIKA-GAKU" — *THE PEDIATRICS, ESPECIALLY CHILD-CARE AND TREATMENT* —, P. 15-17, BY KARASAWA, K. IZUMI, N.; NANZANDŌ, TOKYO, 1940.

TABLE XV

IN VITRO AND IN VIVO VALUES FOR 838 TOXOID (COMPARATIVE DATA ON OTHER DIPHTHERIAL TOXINS)

TOXIN	L.R./100	(L.R.)*	MLD	L.F. (DOSE)
TORONTO NO. 2	0.00046 ml.	0.046 ml.	0.0034 ml.	0.0312 ml.
1 YEAR OLD TOXIN NO. 10-K	0.00106 ml.	0.106 ml.	0.1 ml.	0.077 ml.
GRAVIS NO. XXI	0.004 ml.	0.4 ml.	0.0167 ml.	0.02 ml.
MITIS NO. 402	0.009 ml.	0.9 ml.	0.01 ml.	0.0167 ml.
TOXOID NO. 838	0.01 ml.	1.0 ml.	0.1 ml.	0.5 ml.

* CALCULATED ON BASIS OF L.R./100 DOSES USING DILUTION RATIO 1:1.

TABLE XVI

the 30th and 40th day. Data were not available on eight other deaths. In Table XIV the average ages in months of the children in each lethal time group are given. For the 35 day group the average is 17.5 months. Because information on weights from autopsy protocols and clinical histories were conspicuously missing, weights on these children must be computed from standard weight tables. Such data are given in Table XV. The ratio of females to males for this group was 4 ; 5, so that the average weight was 9.7 kilograms. Thus, when the MLD for 250 gram guinea pigs, calculated on the basis of 720 hour maximum lethal time, was 0.04 ml. of toxoid, the MLD for children weighing 9.710 kilograms was 1.0 ml. of the same toxoid within the same lethal time.

3. The Amount of Toxin in the Tested Toxoid - Of academic interest (at the time of the study) was the matter of how much antitoxin was needed to neutralize the toxic fraction of Lot No. 8 toxoid. Lots of toxin prepared from various diphtheria bacilli recovered from cases in the Tokyo area were tested along with the Lot No. 8 Toxoid and the results are shown in Table XVI . At the time these assays were carried out only National Drug Commercial antitoxin was available as a tertiary standard for calculating the Lf value of Lot No. 8 toxin-toxoid. (The Lf, or flocculation value of a toxin represents the amount of toxin that gives initial flocculation with one unit of antitoxin and is expressed as the reciprocal. It is expressed on a per ml. basis, and in the case of the present titration, includes both the toxoid and the unneutralized toxin). The value of the Osaka product 838 was 2 Lf/ml. In this particular antitoxin the flocculating value (in vitro test) and the protective value (in vivo test) were approximately equal, and 1 Lf unit is roughly equivalent to one standard antitoxin unit (a.U.).

Using secondary standard antitoxin, the Lr/100 dose of the Osaka product was found to be 0.01 ml. The full Lr dose ("the least amount of toxin which, when mixed with one unit of antitoxin, forms a mixture of which 0.2 ml. injected intracutaneously into guinea pigs causes a minimal skin reaction") is therefore 1.0 ml., and the amount of toxin in 1.0 ml. is greater than the amount of toxin completely neutralized by one antitoxin unit.

Since in vitro tests showed that the total flocculating material (toxin-toxoid) per ml. was equivalent to 2 Lf. units, and since in the particular antitoxin used, this is the equivalent of 2 A.U., it may be safely stated that more than half of the specific, immunogenic (as indicated by Ramon flocculation) corynebacterial metabolites in the tested toxoid was diphtherial toxin.

4. Formalin Content - Using methods described in "Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists", 6th Edition, a negative reaction for formalin was obtained on one ampule of Lot No. 8 toxoid. A similar test on American toxoid gave a value of 0.17 mgms/ml.

5. Discussion - Unofficially, seven hundred children have been reported as having suffered some ill effects from the toxoid evaluated in this report. Sixty-two of these children have died. The majority of the deaths may be attributed to the diphtherial toxin contained in 1 ml. of the Osaka toxoid. Since all of the children had, from five to seven days previous to the fatal doses, received initial doses of toxoid, they undoubtedly had the advantage of some, if very small, immune response with which to meet the second and toxic dose. The Lf value of the first toxoid is not known. Regardless of its exact value, 10 guinea pig MLD, in the face of some immunity on the part of a child recipient, was enough diphtherial toxin to kill a child of 9.710 kg. weight in 35 days. Proof of existing vestigal immunity on the part of these children would merely serve to lower the MLD's comprising the LD. Interesting, in relation to the just stated LD value, is one of the more recent assertions regarding theoretical values for diphtheria toxin in terms of human lethal doses: "Those data which are available indicate that the susceptibility of a child to diphtherial toxin is, weight for weight, about ten times that of the guinea pig. The fatal dose for the child would, therefore, probably be less than ten guinea pig MLD." (12a.)

6. Summary - (1) Diphtherial toxoid suspected of causing death in 62 infants was found to have an Lf value of 2 units per ml. (very low for an immunizing agent) and to contain 10 MLD per ml. (2) The human lethal dose in these cases was found to be 10 guinea pig MLD or less.

Certain Toxin Producing Hemolytic Corynebacteria Occurring in Lesions of Man - In a report concerning the bacteriology of diphtheria in U.S. troops in the Kyoto and Kure areas in the winter of 1945-46 (13) mention was made of the incidence of certain hemolytic corynebacteria which produced toxins not neutralized by diphtheria-antitoxin. Liebow and his coworkers (14) indicated experiences with similar organisms in an account of the bacteriology of cutaneous diphtheria in troops in certain areas of the South and Central Pacific. In a subsequent paper (15) devoted to detailed descriptions of their organisms these workers proposed to tentatively designate it Corynebacterium hemolyticum. They suggested that C. hemolyticum bore some relationship to C. pyogenes and C. ovis.

Reports of the isolation from man of corynebacteria which produce toxins not homologous with diphtheria antitoxin (15-21) are remarkable in that few such toxins (13, 16, 17) are described as themselves being hemolytic or occurring in association with soluble hemolysins. From 1946 to the present in this section there have been isolated a number of B-hemolytic corynebacteria in the course of routine bacteriological studies of throats, urethral exudates and various cutaneous ulcers occurring in the Japanese and in Americans stationed in Japan. These organisms plus representative strains from the Kyoto-Kure group (13) all produce soluble hemolysins and it is with their general biology that this preliminary report is concerned.

1. Materials - The organism of the "hemolyticum" group used for study include, in addition to strains isolated locally, two strains, 53-W-1 and 53-W-2 (received from Dr. A. A. Liebow) and four strains, Czech I, II, III and IV (received from Dr. Potocka of the University of Prague). Those strains of C. pyogenes employed for comparative study were obtained from Dr. Reginald Lovell (22), Royal Veterinary College, Berkshire, and Dr. I. A. Merchant (23), Iowa State College. All strains of C. ovis were received from Dr. Carne (24), of the University of Sidney. Strains of C. ulcerans (20), Dr. Liebow procured for us from the Division of Laboratories and Research, New York State Department of Health, Albany. Standard strains of C. diphtheriae gravis, mitis and intermedius were received from AMDR&GS and bear the numbers 53-A-7, 53-A-4 and 53-A-9 respectively. The strain of P.W. 8 - Toronto used was received from A. M. Pappenheimer Jr. Strains of C. diphtheriae minimus and two strains of a sucrose positive diphtheria toxin producing bacillus were received from Dr. Martin Frobisher (25). It was not possible to obtain the Parker (21) strains. Inclusion of the Barratt strains (18) was not considered necessary.

C. pyogenes toxin and antitoxin were received from Dr. Reginald Lovell. Dr. Carne furnished his 1933 (26) C. ovis antitoxin. All diphtheria antitoxin used were from commercial sources and were checked against secondary standard antitoxin for labeled potency and for in vitro in vivo values. For all in vitro titrations, Wyeth serum was used, since, of the available Army supplied sera, it exhibited the most suitable degree of avidity.

Penicillin used was commercial penicillin standardized against working standard received from U.S. F.D.A. Streptomycin used was that of Merck & Company and Sulfadiazine was a U.S.P grade manufactured by Squibb and Company. Glucose - 1 - phosphate was supplied by the Wahl-Henius Institute and all other chemicals used were of C.P. grade.

2. Preliminary Studies - Hemolytic corynebacteria isolated from different cases offered a heterogenity of morphological and biochemical characteristics; often organisms from the same initial isolation failed to give successively consistent biochemical reactions. This condition became less confusing, however, when several strains under careful study exhibited an ability to exist in two colonially distinguishable forms. These phases were referred to as S and L, Small and Large. Without too much difficulty, dissociation from S to L could be induced; the reverse at this writing, has not been accomplished. Each phase has been relatively stable on blood, serum or egg media. Each exhibits a unique biochemical pattern and it has been necessary, therefore, to maintain them separately. For example, 637-S and 637-L are the L and S forms of a hemolytic corynebacterium isolated in conjunction with C. diphtheriae from a clinically diagnosed case of diphtheria and 14-1-S and 14-1-L are numbers ascribed to the S and L forms of a culture of C. pyogenes received from Dr. Lovell.

In carrying out detailed experiments designed to give data for a more particular comparison between C. pyogenes and those organisms termed "hemolyticum" it was necessary to choose a few typical members of each group. As a representative strain of C. pyogenes the two phases of 14-1 of Lovell were chosen. The type culture of C. hemolyticum, 53-W-1, could not be used for all experiments since it existed only in the L phase; so the locally isolated strain 637 was selected as representative of the "hemolyticum" group.

In Table XVII certain of the morphologic characteristics are set forth for comparative purposes. C. ulcerans and C. ovis are included because some authors (15, 22) have suggested a relationship between these organisms and those of the pyogenes group. The basal medium contained 1% human serum which had been inactivated by heating at 65° C. for one hour. All readings were completed seven days after inoculation. Initial inoculum consisted of 0.01 cc. of a 1% suspension of washed 18 hour harvests from blood plates. Because of the varying proteolytic activity of the pyogenes - hemolyticum group of organisms, it is felt that more accurate results might be obtained using basal medium free of any complex proteins. A carbohydrate base distributed in tubes containing charcoal sacs is now being investigated.

Table XVII. Comparative Morphology

Cells from 6 hour old inspissated serum slants of:	Measurements (Micra) of Gram stained cells	General Morphology	Alkaline Methylene Blue, 20 minutes
C. pyogenes			
14-1-S	0.5 to 2.0	Wedge shaped	Even staining
14-1-L	2.0 to 8.0	Tapering some with lateral protuberances	Even staining
C. "hemolyticum"			
637-S	0.5 to 2.0	Wedge shaped	Even staining
637-L	2.0 to 10.0	Tapering, some branching	Even staining
C. hemolyticum			
Czech. I	2.0 to 10.0	Evident branching	Even staining
C. ulcerans[x]	0.1 to 1.0	Coccoid	Even staining
C. ovis[x]	0.1 to 1.0	Coccoid	Even staining

3. Colonial and cell morphology and staining properties of 14-1 and 637, phase S and phase L. - From 18 hour old Loeffler's slants S forms appear as small, Gram positive rods ranging in shape from coccoid forms to wedge-shaped bacilli. Singly, these bacilli resemble the "flower petal"-like cells mentioned by Lovell (22) in his discussion of the morphology of C. pyogenes. Often two cells occur with their larger ends abutting, giving the configuration described as double "suppository form" by Liebow, et al (14). L forms of both 637 and 14-1 also appear Gram positive at 18 hours on Loeffler's medium. They show a marked polymorphism, large (6 micra long) club-shaped organisms being not uncommon. Cells exhibiting rudimentary branching occur, especially in older cultures. All organisms studied seem variable in reaction with the Gram stain after 24 hours. Films stained for 20 minutes with aged, alkaline Methylene Blue failed to exhibit reddish granules ("metachromatic granules") for any of the strains examined. In various diphtheria bacilli, examined at the same time, the minimus type excepted, excellent reddish granules were demonstrated. None of the organisms examined retained Fuchsin following acid alcohol decolorization in the Ziehl-Neelsen stain. From lesions produced in guinea pigs following intracutaneous injections of 637-S, 637-L, 14-1-S, 14-1-L, 1308-S, 13081-L and 53-W-1 (an L form) smears were made. All of the S forms were predominantly Gram positive and ranged from spheroids to slender bacillary shapes. The L forms were Gram positive and occurred in aggregates measuring up to 12 micra across. On blood plates the S-colonies of C. pyogenes (14-1, Lovell) and C. hemolyticum (637, this laboratory) are just visible at twenty-four hours and are surrounded by noticeable zones of hemolysis. Such hemolytic zones may attain a diameter of 1.5 mm. L-colonies in the above mentioned incubation time assume diameters of .75 to 2.0 mm. with zones of hemolysis 1 to 1.5 mm. greater in diameter than the colony.

Wet mount preparations - In examinations of suspensions from 18 hour old serum broth cultures, using dark ground illumination as well as ordinary lighting, the cells of S forms appear as small rods, 0.5 to 2.5 micra in length, are usually in aggregates of several cells; polymorphism is not very apparent. The L forms range from 1 - 5 micra in length, occur singly and in large aggregates and are markedly polymorphic. Some cells appear as swollen spheres with protruding tubes. These are probably the

swollen club forms of the stained preparations. Others show rudimentary branching; that is, at right angles to the long axis of the cell are one to several lateral protuberances. In at least one case, 14635-R, branching was so elaborate as to enable one organism to cover several high power fields. This variant was derived from 14635-L and may represent a true rough form. Chains of coccoid organisms as described by Brown (27) have been observed in 48 hour old cultures of 14-1-S on Mueller's medium plus Calcium Pantothenate, Tryptophane and Glutamic Acid.

4. Group Biochemical Reactions; Dehydrogenase Activity - Routine fermentation tests for proteolytic bacteria involve certain difficulties when the basal medium must contain serum proteins. The possibility of false reactions from hydrolysis of serum is rather obvious. Goldsworthy and Still (33) have further asserted the dangers of serum amylase in serum carbohydrate broth. Mueller (34) has indicated certain dangers inherent in using commercial soluble starch as a test substrate for starch hydrolysis because of the traces of dextrose present. (By substituting laundry type corn starch for soluble starch in carrying out Leeds typing for C. diphtheriae done in this laboratory, forty otherwise starch positive mitis strains were shown to be starch negative).

Careful consideration of the C. hemolyticum group in terms of the other toxigenic corynebacteria required the execution of the work summarized in Table XVIII. It is evident that all listed organisms of the "hemolyticum" group enzymically are more closely related to C. pyogenes than to any of the other included species.

Proteinase production - By slow digestion of coagulated egg and serum and by the liquifaction of serum-gelatin, the proteolytic activity of these bacteria may be easily demonstrated. In addition, they are able through effecting a rennet like action to curdle milk and thereafter to successfully lyse the curd. Brown (27) has shown that the curdling action for C. pyogenes requires free calcium ions for activation. This has been confirmed in this laboratory for both C. pyogenes and C. "hemolyticum". Tests designed to check the susceptibility of various protein substrates to the proteolytic action of crude cell free enzymes have been initiated. Some success has been experienced using the muscle protein technique of Todd.

Phosphorylase activity - Hehre (35) has shown that C. diphtheriae is capable of synthesizing a starch like material from Glucose - 1 - phosphate and suggested that such ability might be used as a differential characteristic for that organism. Here, experiments thus far indicate that similar abilities are shared by the pyogenes group.

5. Certain Physical and Physiological Differences between the S and L Phases - In 18 hour old broth cultures the S forms grow as a diffuse suspension; the L forms as a granular sediment. When washed S phase cells are suspended in 0.85% salt solution of pH 6.8 they remain suspended; L phase cells quickly settle to the bottom of the suspending fluid. All authors in writing about C. pyogenes and C. "hemolyticum" have pointed out that these organisms either "require" blood and/or serum for growth or that they "grow better" with blood and/or serum. Brown (27) pointed out that C. pyogenes was not hemoglobinophilic and observed that its growth was "favored" by "higher protein material such as egg albumin, serum or blood." Charcoal has been found, in this laboratory, to serve as effectively as serum or egg albumin in enhancing the growth of the strains of C. pyogenes and C. "hemolyticum" studied. L-phases grow better in the absence of charcoal or serum than do the S-phases.

The Charcoal Effect - When S and L phases of either of the two species under discussion were inoculated into broth containing dextrose, Tryptose, Difco, and sodium chloride, the S forms showed the only minimal growth at 24 hours; the L forms yield moderately good growth. When cellophane sacs containing 12 mg. of activated charcoal (pretreated with HCl and several times washed with distilled water followed by final washings in buffered distilled water) were introduced into tubes containing 2.5 ml. of the above medium, the growth becomes luxuriant for both strains, the cells being massed about the periphery of the sac. Whether or not the growth promoting effect of the charcoal rests upon the removal to its surface of minute amounts of any or several of the long chain fatty acids which might be in the medium, has not as yet been completely investigated.

Oxygen requirements - Both S and L phases grow well under anaerobic as well as aerobic conditions; S forms doing best under microaerophilic conditions.

6. Group Hemolytic Activity - Hemolysis and Lovell's Toxin: Both C. pyogenes and C. "hemolyticum" produce zones of hemolysis on blood agar. Lovell (26) described a hemolytic toxin obtained from filtrates of broth cultures of C. pyogenes. Samples of his toxin and antitoxins are available. Liebow

	Dextrose	Maltose	Sucrose	Dextrin	Starch	Mannite	Manite	Trehalose	Sorbitol	Levulose	Galactose	Xylose	Inositol	Glycerol	Serum	Gelatin	Egg	Hammerschmidt
Corynebacterium diphtheriae																		
gravis (53-A-7)	≠	≠	-	≠	≠	-	-	-	-	≠	≠	-	-	≠	-	-	-	-
mitis (53-A-4)	≠	≠	-	≠	-	-	-	-	-	≠	≠	-	-	≠	-	-	-	-
intermedius (53-A-9)	≠	≠	-	≠	-	-	-	-	-	-	-	-	-	-	-	-	-	-
minimus (NS 810,808,832,1051)	≠	≠	-	≠	-	-	-	-	-	≠	≠	-	-	-	-	-	-	-
sucrose fermenter (NS 499,502)	≠	≠	≠	≠	-	-	-	-	-	≠	≠	-	-	≠	-	-	-	-
Corynebacterium ulcerans																		
(NYSDH 170,37142,39164)	≠	≠	-	≠	≠	-	-	≠	-	≠	≠	-	-	-	-	≠	-	-
Corynebacterium ovis																		
(CSIR 1)	≠	≠	-	≠	-	-	-	-	-	≠	≠	-	-	≠	-	≠	-	-
Corynebacterium xerosis																		
(53-K-1)	≠	-	≠	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Corynebacterium pyogenes																		
Lovell (14-1-S,14-1-L) (14-2[x],-3[x],-7[x],-8[x],-9[x]) (S)	≠	≠	≠	≠	≠	≠	-	≠	≠	≠	≠	≠	≠	≠	≠	≠	≠	≠
[x](Organisms used as received. Not separated into S and L.) (L)	≠	≠	-	≠	-	≠	-	≠	-	≠	≠	-	≠	-	-	-	-	≠
Merchant (C-7,-11,-18,P-14,S-26	≠	≠	≠	≠	≠	≠	-	≠	-	≠	≠	≠	≠	≠	≠	≠	≠	≠
Corynebacterium "hemolyticum"																		
(53-W-1)	≠	≠	-	≠	-	≠	-	-	-	≠	≠	-	≠	≠	-	-	-	≠
(53-W-2)	≠	≠	-	≠	-	≠	-	-	-	≠	≠	-	≠	≠	-	-	-	≠
(CZECH I, II, III, IV)	≠	≠	≠	≠	≠	≠	-	-	-	≠	≠	-	≠	≠	-	-	-	≠
(T-14233,-14636)	≠	≠	≠	≠	≠	≠	-	≠	-	≠	≠	-	≠	≠		-		
(K-637,T-15544,-13081, -13853,-14248,-14275, -NK-328,K-637-S,T-13081-S (S)	≠	≠	-	≠	≠	≠	-	≠	-	≠	≠	-	≠	≠	≠	≠	≠	≠
(L)	≠	≠	-	≠	-	≠	-	≠	-	≠	≠	-	≠	-	-	-	-	≠
(T-14635)	≠	≠	-	≠	≠	≠	-	≠	-	≠	≠	-	≠	≠		≠		≠
(J-8,T-15520)	≠	≠	≠	≠	≠	≠	-	≠	-	≠	≠	≠	≠	≠		≠		≠

Table XVIII

Carbohydrate Reactions when 1% of test Carbohydrate is incorporated in a basal medium containing 1% heat denatured serum.

There was no hydrolysis of Dulcitol, Arabinose, Rhamnose, Raffinose.

and his co-workers (15) were not able to produce a soluble cell free hemolysin. Using their strains and those of Lovell it has been possible in this laboratory to produce Seitz filterable hemolysins of high M.H.D. value per ml. when media of ill defined constitution (e.g. Fresh liver Infusion - peptone broth) were used. The use of the partially defined casein hydrolysate medium of Mueller and Miller (28) modified to contain dl-tryptophane, d-Glutamic acid and a maltose-dextrose carbon source is currently being investigated.

Weld serum extraction of hemolysin - Applying the technique of Weld (30) and shaking large volumes of C. hemolyticum cells in 4% serum-saline for twelve hours, low yields of hemolysin were obtained.

Effect of Ribonucleic Acid upon Hemolysin Production - It was not possible to alter the hemolysin yield through the addition to media of Ribonucleic acid (31). Employment of the Ribonucleic acid residual following ribonuclease digestion (32) has not yet been tried.

Production of Antihemolysin - Using in rabbits an immunization routine of primary, secondary and tertiary inoculation of killed cells followed by doses of live cells, it has been possible to appreciably elevate the blocking activity of rabbit serum for these hemolysins.

Agglutinin Content of Antihemolytic Serum - When suspensions of S phase cells of 637, 14-1, P 14, and S-26 are used as antigens against serial dilutions of the antihemolytic sera mentioned above, there is marked cross agglutination. L phase cells cannot be used as antigens for reasons already stated.

7. Corynebacterial Toxins - On the basis of neutralization by antitoxin there apparently is no relationship between the toxins of C. pyogenes and C. diphtheriae or between those of C. "hemolyticum" and those of C. diphtheriae. This is borne out by the results shown in Table XIX. In this laboratory, the technique of Frazer and Weld (36) has been an invaluable tool for picking up toxigenic corynebacteria not related to C. diphtheriae.

Table XIX

Neutralizing Effect of Diphtheria Antitoxin (PW-8)
On Corynebacteria Considered in this Report

	Reaction of guinea pigs to various strains of Corynebacteria under the conditions of the virulence test of Frazer and Weld[x]	
	Reaction when test dose is administered 4½ hours before administration of diphtheria antitoxin.	Control reaction where identical test dose is given 30 minutes following the administration of 750 Lf units of diphtheria antitoxin.
C. diphtheriae gravis (53-A-7) mitis (53-A-4) intermedius (53-A-9)	Necrosis	No reaction to slight flush
C. ulcerans	Necrosis	Necrosis
C. ovis	Necrosis, slight oedema	Necrosis, slight oedema
C. xerosis	Slight flush	No reaction
C. pyogenes	Oedema, central induration	Oedema, central induration
C. "hemolyticum"	Oedema, central induration	Oedema, central induration

x Final readings on both test and control inoculations are made 70 hours following the last control inoculation.

Coprological Studies - Table XX. Breakdown of Significant Enteric Bacilli Isolated

	Mucus Positive Cases		Mucus Negative Cases		Not Noted		Total
	602		885		606		2093
Organisms Isolated	Occupation Personnel	Japanese Personnel	Occupation Personnel	Japanese Personnel	Occupation Personnel	Japanese Personnel	
Shigella							
dysenteriae	5	5	0	0	2	0	12
ambigua	1	3	1	1	5	0	11
sonnei	7	38	4	29	21	0	99
paradysent. W.	4	66	1	28	5	0	104
paradysent. Z.	0	6	1	1	6	0	14
paradysent. Boyd 103	0	1	0	0	5 (1)[x]	0	6 (1)[x]
paradysent. Boyd 88	0	0	0	0	2	0	2
paradysent. Boyd 170	0	0	0	0	1	0	1
sp. Sachs Q771	0	0	0	0	1	0	1
sp. Sachs Q1167	1	1	0	0	1	0	3
alkalescens	1	0	2	0	2	0	5
ceylonensis	0	0	1	0	0	0	1
Salmonella							
paratyphi A	0	0	1	6	7	0	14
paratyphi B	5	0	2	0	4	0	11
paratyphi C	0	0	0	0	4	0	4
typhosa	1	1	0	1	0	0	3
typhi murium	1	0	1	0	0	0	2
enteritidis	0	1	1	0	0	0	2
Group F	0	0	0	0	1	0	1
Paracolon with -							
sh. dysenteriae factor	4	0	0	0	0	0	4
sp. Q771 factor	16	1	1	0	7	0	25
Sh. ambigua factor	0	0	0	0	1	0	1
Salm. para A factor	0	0	0	0	2	0	2
Salm. group factor	0	0	0	0	2	0	2
Proteus Morgani	0	5	4	8 (2)[x]	4	0	21 (2)[x]
Total	46	128	20	74	83	0	351

[x]Double Infections

Coprological Studies - 2,093 specimens were processed during the period of this report. Of these 351 were positive and 1,746 negative. Five strains (ceylonensis and alkalescens) should probably be deleted on the basis of non-pathogenicity. The status of the included paracolons is debatable (see text).

A breakdown of the positive cases comprises Table XX. Information may be derived regarding the occurrence of the different enteric pathogens in this command, but the incidence of these pathogens cannot be gauged since the majority came from the Tokyo-Yokohama area and selected areas in Kyushu. Data on the presence of leukocytes, erythrocytes and or mucus in the stools examined are not complete. The incomplete data have been assembled in a separate column marked "not noted".

1. The demonstration of agglutinins for Shigellae and Salmonellae in fecal extracts as an aid in the laboratory diagnosis of intestinal disorders of bacterial origin - Harrison (37) on the basis of work carried out by Burrows (38) on fecal agglutinins in experimental enteric cholera in guinea pigs, initiated a study of fecal agglutinins in humans suffering with salmonellosis, shigellosis and chronic ulcerative colitis. He reported, as had Davies (39) and the Russian workers (40, 41, 42) that the demonstration of fecal agglutinins seemed to offer a valuable diagnostic aid, especially in cases (chronic ulcerative colitis) where no etiologic agent was recovered. Work in this laboratory on coproagglutinins indicates that, while their demonstration in feces offers useful data concerning the immune response of the patient. the demonstration of their presence per se is not necessarily of specific diagnostic value.

Method - (1) The fecal sample was matched with an equal volume of formol-saline (1:10,000 formalin in 0.85% saline) until microscopic examination and inoculation on selective and non-selective media could be effected.

(2) After inoculation the remaining portion of the fecal specimen was thrice frozen and thawed. The suspension was then centrifuged and the supernatant tested for the presence of copro-agglutinins (this liquid is referred to hereafter as the "fecal extract").

(3) Agglutinations using 1/5 dilutions of the extract, were set up against Escherichia coli 97-C-K, an indigenous E. coli antigen and an antigen prepared from the isolated pathogen and from the stock homologue of the isolated pathogen. (In an earlier report (this laboratory, Technical Report to the S.G.O., October 1948) a 10 hour broth culture of the specimen was employed vice E. coli 97-C-K. The rationale behind the substitution of 97-C-K will be found in part 2, below).

(4) Specimens were collected on cases as long as it was possible to retain the patients.

In Tables XXI, XXII, XXIII, XXIV four types of cases are presented. The data are fairly self-explanatory. The presence or absence of agglutination with the fecal extract is shown by + or -. The term "indigenous" refers to organisms obtained from the particular case under study.

Table XXI. S. paradysenteriae W

Case A

Extract	Date	E. coli 97-C-K	Indigenous Flexner W antigen	Stock Flexner W antigen	Indigenous Esch. coli antigen	Recovery of Indigenous pathogens	Gross & microscopic
25	18 Aug.	+	+	+	+	Yes	MPB[x]
40	23 Aug.	+	+	+	+	Yes	MPB
47	25 Aug.	+	+	+	+	Yes	MPB
71	2 Sept.	+	+	+	+ vy.wk	No	MP
97	7 Sept.	+	+	+	+ wk	No	-
117	10 Sept.	+	-	+	+	No	-

x mucus, pus and blood

Table XXII. Shigella dysenteriae

Case B

Extract	Date	E. coli 97-C-K	Indigenous Sh. dysenteriae	Stock Sh. dysenteriae	Indigenous Esch. coli	Recovery of Indigenous pathogens	Gross & microscopic
56	31 Aug.	≠	≠	≠	-/	Yes	MPB
69	2 Sept.	≠	≠	≠	≠	Yes	MPB
87	4 Sept.	≠	≠	≠	≠	No	MP
88	6 Sept.	≠	≠	≠	≠	No	MPB
102	8 Sept.	≠	≠	≠	≠	No	MP
122	13 Sept.	≠	≠	≠	-	No	MPoc Boc
134	15 Sept.	≠	≠	≠	-	No	Poc
142	17 Sept.	-	-	≠	-	No	-
150	20 Sept.	-	≠	-	-	No	-

oc - rare

Table XXIII. Shigella sonnei

Extract	Date	97-C-K	Indigenous Sh. sonnei	Stock Sh. sonnei phase I phase II	Indigenous Esch. coli	Recovery of Indigenous pathogens	Gross & microscopic
52	25 Aug.	≠	-	≠ wk	-	Yes	P
61	31 Aug.	-	≠	-	-	Yes	MP
65	2 Sept.	-	≠ wk	-	-	Yes	M
82	4 Sept.	-	≠ wk	≠ wk	-	Yes	Poc
90	6 Sept.	-	-	-	-	Yes	MP
103	8 Sept.	-	≠ wk	≠ wk	-	Yes	Poc
113	10 Sept.	-	-	-	≠	Yes	Poc
124	13 Sept.	≠	-	≠ wk	≠	Yes	Poc
136	15 Sept.	≠	-	-	≠	No	-
144	17 Sept.	≠	-	≠	≠	Yes	-
152	20 Sept.	≠	-	≠	≠	No	MPoc

Table XXIV. Coliform with Q771 Antigens

Case D

Extract	Date	97-C-K	Indigenous Pathogen	Stock A.M.D.R. & G.S. Q771	Recovery of Indigenous pathogen	Gross and Microscopic
39	23 Aug.	-	+	+	No	-
51	25 Aug.	+	+	+	Yes	-
60	31 Aug.	+	+ wk	-	No	P
64	2 Sept.	+	+	+	Yes	P
81	4 Sept.	-	+	-	No	-
89	6 Sept.	-	-	-	No	M.P.

Tabular data such as the above have been charted and studied for several hundred dysenteric stools and the following opinions are currently held regarding the laboratory diagnostic value of fecal agglutinins.

(a) In cases of shigellosis and salmonellosis where a possible common source of infection was demonstrated and an incriminable pathogen was isolated from some patients and not from others the demonstration of fecal agglutinins in the stools of some of the culture negative cases might serve as circumstantial evidence of epidemiological value.

(b) In cases of dysentery from which are isolated an unknown organism suspected of being the causal agent, the demonstration of fecal agglutinins against that organism might strengthen the case for its pathogenicity.

2. Agglutinins for E. coli in fecal extracts - During the course of study of 1048 fecal extracts from cases of acute bacillary dysentery it was observed that agglutinins for the isolated Shigellae often occurred concomitantly with agglutinins for certain coliform organisms, namely strains of E. coli, Aerobacter aerogenes and Proteus vulgaris. That these coliform agglutinins were separate, specific and in no way associated with the para-agglutination phenomenon of Mackie (43) and others was established by two means: (1) antiseral screening of the organisms for the absence of Shigella factors and (2) the demonstration of strong coliform agglutinins in extracts rendered non-reactive for Shigella factors by absorption with Shigella antigens. As this study proceeded, it became evident that while agglutinins were demonstrable against some members of the indigenous coliform flora in many cases of acute bacillary dysentery, agglutinins did not occur for just any member of the flora. Since working with innumerable coliform antigens greatly complicated the work, an effort was made to select some organisms or organism which might serve as composites for several antigenic types. Sera for factor analysis of the coli group such as has been suggested by the work of Kaufman (44) was not available so the following makeshift method was employed. Strains of Escherichia shown to be agglutinable by their homologous fecal extracts in dilutions of 1/5 or greater, were used for the production of smooth antigens. A large number of these antigens were set up against heterologous extracts from cases of bacillary dysentery attributable to various Shigellae. Some antigens reacted only with their homologous extracts, some with one or two heterologous extracts and one strain, 97-C-K, reacted strongly with extracts from fifty different cases of bacillary dysentery. It was assumed that of the coliform organisms tested, 97-C-K offered the broadest coli factor pattern. It was further demonstrated that this organism possessed no agglutinable factors in common with presently described Salmonellae or Shigellae. Of the organisms tested, then, 97-C-K seemed most suitable for assaying the significance of coli agglutinins in sera and fecal extracts of dysenteric and non dysenteric individuals. Such assay datum is essential if the apparent immune response to coliform organisms in dysentery is to be understood.

Sera from 50 non-dysenteric soldiers and sera from 50 dysentery cases were set up against whole antigen and O-antigen of 97-C-K. Alcohol extracted O-antigen was employed to correct for any clouding of results due to the presence of alpha antigen reactions. The results revealed that agglutinins

for 97-C-K "O" and "H" occurred in demonstrable concentrations in some serum from both groups.

Further work on this problem will be reported later.

3. Shigella Factors in coliform organisms - The observations of Mackie (43) that coliform organisms often possess Shigella antigenic factors has many times been confirmed and extended in this laboratory. There have been isolated here coliform organisms with antigenic factors in common with Shigella dysenteriae, Shigella paradysenteriae W, Shigella paradysenteriae Z, Shigella sp. Sachs Q771, Shigella ambigua, Shigella sp. Sachs Q1167 and Shigella sp. Sachs Q1030. These factors are all heat stable and present in quantities enough to give strong positive reactions with factor specific sera.

At least two of these organisms are unique in that they were isolated from cases of dysentery from which agents of recognized pathogenicity were also recovered. One, an organism (ES791) having at least one Shigella dysenteriae factor was recovered from a frank case of amoebiasis, by all laboratory tests uncomplicated by the presence of Salmonellae or Shigellae. The other, 98-A-K, was a coliform bacillus isolated from a case of Shigella dysentery in which Shigella paradysenteriae W was repeatedly incriminated following bacteriological and serological testing, of the patients' feces. In all probability, then, these two organisms did not cause dysentery and their presence in dysenteric stools is probably no more significant than were the organisms described by Mackie (op. cit). The primary interest in them had to do with their relation to fecal agglutinins. Their serologic community with pathogenic Shigellae has been mentioned. By mouse protection tests, summarized in Table XXV their immunogenic value as sources of vaccine against Shigella dysenteriae, in the case of ES-791, and against Shigella paradysenteriae W in the case of 98-A-K was also established. Further information about such organisms should serve to more clearly delineate the serological relationships and patterns of existing Shigella species. For example, because of the one sided appro ch to the serology of the Shigella group, e.g. establishing their pathogenicity, giving them admission to a small group and then establishing intrageneric serological distinctions for the exclusion of future members, only those antigenic factors can be discovered which are demonstrable through reactions involving various homologous and heterologous combinations of antigen and antibody within the group. In all probability, many more factors might be discerned were the boundaries for serologic testing not so limited. The field of factor significance, which has barely been scratched, would thereby gain a number of new tools for assay.

Table XXV

Coliform Vaccine	Challenge Pathogen	Number of M.L.D. Withstood
98-A-K	Shigella paradysenteriae W	43 M.L.D.
ES-791	Shigella dysenteriae Hanabusa	17.6 M.L.D.

4. Coliforms with Sachs Q771 factor - Periodically over the past two years this laboratory has reported coliform bacilli with Q771 antigens. Not until the past summer, however, did this organism (or these organisms) occur in significant case numbers and with symptoms dramatic enough to justify its (their) being seriously considered as an enteric pathogen. On 13 August, 1948, 72 E.M. reported on sick call at the 385th Dispensary (Finance Branch) with complaints of gastrointestinal disturbance. Between 13 August and 9 September this Section received 26 stool specimens on an equal number of people from the complaining group. From 21 of these 26 specimens the coliform organisms in question were recovered. Careful follow-up work was not effected on these cases. In December of the same year several members of an American Mission to Japan were stricken with dysentery 24-30 hours following the attendance of a special dinner. Stool specimens were submitted on two of these individuals. From one case who suffered a protracted and moderately severe dysenteric episode the coliform under question was isolated in almost pure culture. In Nagasaki-ken (Kyushu) a 3 year old child with dysentery was carefully studied by members of this Section. The same coliform was the only significant organism isolated from the child and strong fecal agglutinins for the Q771 factor were demonstrated. More recently, from the 49th General Hospital in Tokyo one of these organisms was referred for identification. While related stool specimens were not available for study and initial microscopic examination had not been made, the clinical history revealed that the case, a 6 year old child, had suffered from watery diarrhea for seven days.

Coliform bacilli with the Sachs Q771 factor have been isolated from 24 cases. None of these cases were complicated by the presence of known enteric pathogens. In 17 of these cases microscopic examination was performed; the stools contained mucus in 16, leukocytes in 15, and erythrocytes in 11 instances. All but two of the paracolon bacilli collected from the above cases are typified by this laboratory's culture No. ES-193, a non-motile, non-sporulating, gram negative, short bacillus capable of readily hydrolizing dextrose, mannite, arabinose, xylose, dulcite and slowly hydrolyzing lactose with the production of acid and gas, but not capable of effecting the hydrolysis of sucrose, raffinose, inosite or salicin. Indole is produced. Voges-Proskauer and Methyl Red tests are negative. Citrate is not utilized, there is no evidence of urease activity. The organism possesses a heat stable antigenic factor which reacts strongly with absorbed sera prepared by AMDR&GS against Shigella sp. Sachs Q771. Complete removal from this sera of agglutinins for Sh. sp. Sachs Q771 is possible by absorption with ES-193. When ES-193 homologous serum is absorbed with Sachs Q771, the serum becomes negative for both ES-193 and Sh. sp Sachs Q771.

The one exception to the general pattern set down above for ES-193 is an organism designated in this laboratory as ES-142. A slow lactose fermenter, it was isolated from the feces of a patient suffering from dysentery. The organism was referred to this laboratory for study. Serologically, it exhibits an attribute unique in the group under consideration; it possesses a heat labile, agglutinin blocking antigen. ES-142 is sluggishly motile and utilizes citrate. Antiserum against it is necessary before its exact position in the above described group may be ascertained.

5. Salmonella paratyphi B in immunized groups - Salmonella paratyphi B was recovered from one man from a large enlisted billet on 30 November. A day later an organism submitted for typing from the 49th General Hospital Annex also proved to be an S. para-B. Both men lived in the same billet; both had complained the day following Thanksgiving. One had been sick enough for hospitalization; the other not. In each case illness was of no more than two days duration. The two cases were interrogated and it was discovered that each had a friend who had experienced intestinal disturbances 1½ to 2 days following Thanksgiving. Cultures of these later two yielded S. paratyphi B. Subsequent culturing of three of the four at various periods resulted in the isolation of the same organism. Fecal extracts agglutinated the recovered organism at one time or another in each case. In Table XXVI data complete to the time of this report are given relative to the bacteriological and serological findings on feces examined from three of these persons. More definitive serological data comprise Table XXVII, where it is demonstrated that agglutinins for flagellar antigens a (S. paratyphi A), d (S. typhosa) were present as well as those for b, 1 - 2 (S. paratyphi B). There is a distinct difference in concentration, however, and the difference is in favor of the isolated pathogen, S. paratyphi B. Case A received his last T.A.B. recall dose in early November, 1948; Case B's date of recall inoculation was also November; Case C's was May, 1948. The respective serum titers for T.A.B. are offered in Table XXVIII.

Table XXVI. Recovery of S. paratyphi B From Each Submitted Fecal Sample as Compared with the Demonstration of S. paratyphi B Specific Fecal Agglutinins

		Nov.			Dec.						Jan.		
		30	1	2	5	7	9	11	18	20	22	31	5	6
Case A	Culture		P		N			N		N	P		N	
	Agglutinins		N		P			N		P	P		N	
Case B	Culture	P				P	N	N				P	N	
	Agglutinins	P				P	P	P				P	P	
Case C	Culture			P		P			N	N				P
	Agglutinins			N		P			P	P				P

(P = Positive, N = Negative)

More work must be carried out on these and similar cases before any valid conclusions may be drawn. It is interesting to observe that agglutinins for the entire T.A.B. group are excreted in these cases and further that the employment of what amounts to agglutinin typing is necessary in order to ascertain which agglutinins are of diagnostic significance. Further information on a larger group of cases should throw some light on whether or not the secretion of fecal agglutinins in such cases might serve to enhance the possibility of subsequent carrier states obtaining.

Table XXVII

In Which One Fecal Extract Most Reactive Against S. Para - B From Each Case Is Assayed Against Flagellar Antigens of S. Para A, S. Para B and S. Typhosa

Extract	Flagellar Antigens S. Para A	S. Para B	S. Typhosa
Case A	2/	3/	-
Case B	2/	4/	2/
Case C	/	2/	-

Table XXVIII

Serum Titers for Components of Typhoid Vaccine

Serum Samples Taken on 8 Dec.	Indigenous S. Para B "H"	Pathogen S. Para B "O"	AMDR&GS S. Para B H	AMDR&GS S. Para B O	AMDR&GS S. Para A H	AMDR&GS S. Para A O	AMDR&GS S. Typhosa H	AMDR&GS S. Typhosa O
Case A (Nov. '48)[X]	640	160	640	40	160	40	160	0
Case B (Nov. '48)[X]	640	320	640	160	160	40	320	160
Case C (May '48) [X]	640	320	320	80	80	20	320	0
Serum Samples Taken on 7 Jan.								
Case A	320	0	640	0	40	0	160	0
Case B	160	40	320	80	80	20	160	40
Case C	320	40	160	160	40	40	320	80

[X] Date of last inoculation with T.A.B. Vaccine

Endpoints: Final Tube Showing 2/ Agglutination

6. Shigella dysenteriae infections in troops - The first known cases of U. S. troops with Shiga infections in Occupied Japan were demonstrated in December. From a specimen received from the 5th Station Hospital on 3 December, Shigella dysenteriae was isolated. Investigation of the case revealed that the patient, an enlisted man, had been passing bloody stools since 27 November. This man had arrived in Japan on 25 November, landing in Yokohama and proceeding to the Replacement Depot where he ate Thanksgiving dinner.

Subsequent to the discovery of the above case, a total of 7 more suspect cases was found at the Replacement Depot and the adjoining Station Hospital. While Sh. dysenteriae was recovered on only four of these, fecal agglutinins specific for it were demonstrated in extracts from all seven cases; strong fecal agglutinins for E. coli 97-C-K were also noted.

Some of the strains of Sh. dysenteriae recovered from these cases were singular in that they were capable of showing some growth on S.S. Agar. One strain, ES-136, possessed other very singular traits. Replating on veal infusion agar of isolates from selective media yields three distinct types of colonies: translucent (T), opaque (O) and translucent-opaque (T-O). T-colonies reproduce T colonies, O-colonies reproduce O and T-O colonies produce both T and O colonies. The biochemical reactions of these dissociants may be found in Table XXIX.

Table XXIX. Biochemical Reactions of Shigella dysenteriae Isolates

	Control	Test		
	Sh. dysenteriae Hanabusa	E. S. 136 T	E. S. 136 O	E. S. 136 TO
Dextrose	A^1	A^1	A^1	A^1
Sucrose, Lactose	-	-	-	
Mannite, Salicin	-	-	-	
Trehalose	A^4	A^4	A^4	A^4
Xylose	-	-	-	-
Sorbite, Maltose	-	-	A^4	-
Dulcite, Inosite	-	-	-	-
Arabinose, Dextrin	-	-	-	-
Indol, Citrate	-	-	-	-
MR, VP	-	-	-	-
Motility	-	-	-	-

Note: Inoculum consisted or 0.1 cc. of 18 hour culture of test organism. Exponent number of 24 hour periods lapsing before color change or Brom.-Cresol Purple occurred.

Table XXX. Serological Reactions of E. S. 136

E.S. 136 Colonies	Homologous Fecal Extract	A.M.D.R. and G.S. Sera Absorbed Sh. Dysenteriae	Absorbed Sonnei II
T	/	/	-
O	/	-	/
TO	/	/	-

In Table XXX are serological findings on the E. S. 136 strains. The loss variants, opaque colonies, exhibit an interesting serological picture. The acid agglutination points of these three phases are at present under investigation.

7. Summation - It is apparent that the investigation of fecal antibodies is only in the beginning phases. The potential importance of the immunologic response elicited by organisms, usually considered non-pathogens, during the course of an enteric infection with a known pathogen can be assessed only by continuing observation of human cases, with possible amplification by animal studies. The source of the antibody should be ascertained. At the present time, the work is tedious, consuming of materials and man-power, and will progress slowly.

Plans for 1949

1. A preliminary evaluation of the three lysin assay of sera from Rheumatic Fever, Scarlet Fever, etc. has been outlined.

2. The work on the hemolytic corynebacteria will be completed.

3. Studies on copro-antibody will be extended and preliminary report submitted for publication.

4. Work on enteric bacteria significant in disease will be continued.

5. The investigation of an aerogenes-klebsiella group organism, including establishing its role as etiologic agent in an epizootic among guinea pigs, will be completed.

6. Work on the in vitro/in vivo values of antitoxins, prepared in rabbits and horses, against toxins, produced on synthetic media, of C. diphtheriae gravis, mitis and intermedius will be completed soon.

7. Further improvements on methods for the laboratory diagnosis of tuberculosis are anticipated.

PATHOLOGY SECTION

Under the provisions of AR 40-410 and GHQ, FEC Circular 69, 1947, this section functions as a histopathology center for all hospitals in Japan, Korea, and the Marianas-Bonin Command. Histopathologic service as required by other sections of this laboratory is also furnished.

Routine

During 1948 the section has been responsible for the work-up or review of pathologic material under the following major classifications:

Human Autopsies	393
Human Surgical Pathologic Examinations	2709
Miscellaneous Examinations	2245
Total	5347

This represents a 32% increase in volume of examinations over 1947.

Human Autopsies - The Far East Command policy of evacuating to the Zone of the Interior all transportable cases with serious illnesses results in a high incidence of violent or unnatural deaths and acute infections in our autopsy series and a low incidence of malignancies and other chronic debilitating disease. Trauma, by far, is the major cause of death in this series.

A tabulation of autopsy material follows:

Traumatics	86	Ethyl alcohol poisoning	4
Gun Shot Wounds	59	Malignant Neoplasms	3
Drowning	35	Bacterial endocarditis	3
Japanese B Encephalitis	33	Acute pancreatitis	3
Coronary sclerosis with or without thrombosis	24	Acute hepatitis of varied types	3
Stillbirths	18	Diphtheria toxoid poisoning	3
Pneumonia	13	Procaine sensitivity	2
Acute anterior poliomyelitis	10	Diabetes mellitus	2
Prematurity	8	Arteriosclerotic heart disease	2
Hanging	6	Pulmonary tuberculosis	2
Cerebral hemorrhage, spontaneous	5	Intestinal obstruction due to congenital malformations	2
Methyl alcohol poisoning	5	Homicidal stab wounds	2
Complications of appendicitis	5	Acute Cyanide poisoning	2
Encephalitis of unknown etiology	5	Miscellaneous	44
Electrocutions	4	Total	393

The miscellaneous group includes single cases of a variety of conditions. Violence or other unnatural causes accounted for 207 deaths, natural causes, 186. The high incidence of violent or unnatural deaths, all of which must be handled as painstaking medico-legal cases, brings the section into close contact with various line of duty investigating officers and even closer liaison with the Criminal Investigation Division of various Provost Marshals. Relations with the Provost Marshal of the Tokyo Metropolitan Area have immeasurably improved during the year. The resources of the X-Ray Service of the 49th General Hospital and the Chemistry Section of this laboratory have been used fully to thoroughly investigate these deaths and to reduce the number of unexplained deaths in our medico-legal series to an extremely low level. By treating these as medico-legal cases and causing careful search for antecedent disease to be made, the educational experience of the assigned officers has been enriched in one of the most difficult fields in pathologic anatomy.

Twenty-four deaths were attributed to coronary sclerosis with or without thrombosis. Fatal coronary arterial lesions were encountered in the following age distributions:

AGE	NO. OF CASES
19 - 30	4
31 - 40	6
41 - 50	7
51 - 60	7

Among the miscellaneous causes of death were one case of relapsing fever and one case of typhoid fever. Death in the case of relapsing fever occurred in Korea in a 23 year old soldier on the 5th day of illness from massive hemoperitoneum secondary to spontaneous rupture of the spleen. How he acquired the disease is unknown. Death in the case of typhoid fever occurred in a 38 year old civilian in Korea on the 30th day of illness from toxemia as evidenced by high fever, delirium and abdominal distention. The causative organism was isolated. Autopsy in this case showed multiple ulcers of the ileum, acute splenic tumor, focal necrosis in mesenteric lymph nodes, fatty metamorphosis of the liver and petechiae in the kidneys, adrenals and liver. Immunization records indicated that he had received an initial immunization with triple typhoid vaccine six months prior to the onset of illness.

During the 1948 summer epidemic of Japanese B encephalitis autopsy material was received for pathologic examination. The average duration of disease in the 25 cases was 5.5 days and the range of the duration of disease was from 3 to 7 days. The main features of the material reviewed to date are (a) the diffuse encephalomyelitis involving nearly all portions of the cerebrum, brain stem, cerebellum and spinal cord; (b) the perivascular lymphocytic cuffing in the white and grey matter, the high number of neutrophils in the infiltrates in the brain and spinal cord tissue and the formation of glial nodules throughout and (c) the successful isolation of Japanese B encephalitis virus from the 4 cases in which personnel of this laboratory performed the autopsies.

Histologic examination of blocks of tissue from 3 Japanese cases in the Kyoto area who died after receiving diphtheria toxoid has been afforded. In one case, a 6 year old girl dying 10 days after injection, there was extensive cellulitis of adipose and connective tissue presumably from the site of inoculation, liver abscesses and pneumonia. In the second case, a 15 month old girl dying 15 days after injection, there is a toxic type of myocarditis with evidence of aspirated material in bronchioles in the lungs showing congestion and edema. In the third case, a 10 month old boy dying 24 days after injection, there is extensive broncho-pneumonia. In the second and third cases no tissues were received for study from the areas on the arms where the clinical histories indicate there were extensive lesions at the site of inoculation.

Human Surgical Pathologic Examinations - Malignant neoplasms were encountered in 55 specimens of the total of 2709 specimens examined during the year. The types and locations of malignancies are shown in the table below:

Basal cell carcinoma of skin	22	Embryonal carcinoma of testes	1
Squamous cell carcinoma of skin	3	Serous papillary cystadenocarcinoma of ovaries	1
Baso-squamous cell carcinoma of skin	1	Undifferentiated carcinoma of stomach	1
Carcinoma of breast, all types	4	Squamous carcinoma of larynx	1
Adenocarcinoma of cecum	1	Secondary carcinoma in cervical lymph node	1
Adenocarcinoma of recturm	2	Lymphosarcoma of cecum	1
Squamous cell carcinoma of tonsils	1	Myosarcoma involving prostate and bladder	1
Papillary carcinoma of bladder	2	Canalicular sarcoma of breast	1
Squamous cell carcinoma of cervix	2	Fibrosarcoma of scalp	1

A large number of the examinations were appendices, 1203 out of the total of 2709. A high percentage of these showed bona fide evidence of disease. Endometrial biopsies and incomplete abortions accounted for 304 examinations. A variety of dermatoses and benign cutaneous neoplasms accounted for 397 examinations. The amount of time required to work up cutaneous material has been much greater than the number of examinations would indicate due to the difficulty of dermatopathology, particularly when the lesions are rarely seen before removal. Five cases of granuloma inguinale have been encountered during the year.

The adoption of the Starry-Warthin stain for spirochete staining has reduced the time required to work up material in which spirochetes or Donovan bodies are considered as possible etiologic agents. Considerable improvement has been attained in our histologic studies of bone by the adoption of a method using electrolytical decalcification of bone which has reduced the time required for decalcification and given better histologic detail.

Miscellaneous Examinations - The tabulation of miscellaneous examinations below illustrates the scope:

Guinea pig examinations for tuberculosis	1933
Aschheim-Zondek tests	169
Animal brains for rabies	80
Guinea pig examinations for diphtheria studies	2
Rabbits for schistosomiasis	4
Human blood and marrow smears	19
Mouse brains for encephalitis	8
Miscellaneous	22

The number of miscellaneous examinations in 1948 more than doubled those made in 1947 and is equally consuming in man hours labor as our human surgical pathologic examinations.

A positive diagnosis of rabies was established in a much greater number of cases in 1948 than in 1947 reflecting both an actual increase in incidence and improvement in technical work so that the disease would be recognized. During 1948 material from 74 dogs was examined in this laboratory. In 39 cases it was possible to carry out both histopathologic studies of the dog brain and mice inoculations. In 21 cases it was possible to do only histopathologic studies of the dog brain as suitable material for mice inoculations was not submitted. In 14 cases it was possible to do only mice inoculations since fixed tissue was not available for histopathologic studies.

A tabulation of results on these 74 canine brains reveals that a diagnosis of rabies was made 29 times.

Type of Examination	Positive for Rabies	Negative for Rabies	Total
Pathologic and Virologic together	19	20	39
Virologic alone	5	9	14
Pathologic alone	5	16	21
Totals	29	45	74

A correlation of results in the 39 cases in which material was available for both pathologic and virologic studies shows agreement in positive cases 18 times, agreement in negative cases 20 times, negative pathologic results and positive virologic results in one instance and no other instance in which there was disagreement in results.

Liaison

During 1948 material and all available data have been sent to the Army Institute of Pathology in 331 autopsies and 1800 surgical pathology specimens. The large number of surgical pathology specimens reflects to some extent the time consuming screening of 1946 and 1947 surgical material. The screening of this material and preparation of the data to accompany the specimens has cost many man hours labor in 1948. The job is about 75% complete and simply needs additional time for completion.

Relations with the 49th General Hospital and 361st Station Hospital here in Tokyo have been excellent.

There has been a marked improvement in teaching activity during the year. Bi-monthly conferences have been held with the Surgical Service of the 49th General Hospital using our surgical pathologic material and autopsy material to the maximum. Only in rare instances has it been necessary to run a dry conference using material from civilian stateside hospitals. The use of a home made projector helped considerably in building interest and attendance at these meetings. This has been further improved by the acquisition of a Bausch and Lomb microslide projector with which satisfactory presentation of histopathologic specimens is possible. In August a Tumor Board was established at the 49th General Hospital with the Chief of Pathology Service representing Pathology on the board.

A number of clinical pathologic conferences have been presented with the Medical Service of the 49th General Hospital using autopsied cases from their service. A number of clinical pathologic conferences have been arranged for presentation before all services of the 49th General Hospital with the Surgeon General's various civilian consultants in Internal Medicine.

Plans for 1949

It is expected that the large number of cases of Japanese B encephalitis now being worked up from the histopathologic point of view will be completed.

The large number of routine appendix examinations has made it possible to undertake a study with the Medical Zoology Section using parasitologic and pathologic techniques to determine the incidence of protozoal and helminthologic infections in appendicitis and the incidence of these infections in appendices in which no significant lesions are present. The 1948 material will serve as a control.

Material from some of the diphtheria toxoid deaths among Japanese Nationals in the Kyoto area arrived and it is contemplated that data will be assembled for review and publication if feasible.

Efforts will continue to be made to use the available photographic services to illustrate the surgical pathology and autopsy material to enhance the value of clinical records. Preparation of clinical pathologic conferences for the use of hospitals in Japan, Korea and the Marianas-Bonin Command is expected to increase during the year as photographic work improves.

Efforts will be made during the year to raise the standards of autopsies on stillbirths and neonatal cases. This will include correlation of the maternal history and labor record in still births, and these records plus the infant's records in neonatal cases, with the autopsy findings.

Work of the Virus and Rickettsial Section during 1948 was highlighted in the early, middle and late portions of the year by an outbreak of Japanese B encephalitis in Guam, a major epidemic of the same disease in Japan, and to a lesser extent in Okinawa, and the occurrence of tsutsugamushi disease among American military personnel infected in a previously unrecognized scrub typhus area. Much of the data here reported deal with these events. The remaining material covered concerns results thus far obtained in extensive studies on Japanese B encephalitis vaccine evaluation, incidence of inapparent infections among both native populations and occupied personnel, distribution of typhus fever in Japan and other work. Test results are incomplete in some phases of the studies, and in these cases partial data are given; interpretation of the results must await conclusion of the work.

In the course of the past year more than 9,800 serological examinations were carried out including 3,320 virus neutralization tests, 5,140 virus and 1,366 typhus complement-fixation reactions, exclusive of serologic tests required in identification of neurotropic viruses isolated in 1948 from human and animal sources. The Japanese B encephalitis virus complement-fixation test became a routine laboratory test capable of giving specific and highly reproducible results. Modification of the typhus complement-fixation test to include routine employment of soluble antigen has proved its value both for screening tests and for uncovering positive typhus cases failing to give complement-fixation reactions with specific antigens, particularly in the case of murine typhus.

Routine

Complement Fixation Tests for Typhus Fever in Japan, 1948 - Complement fixation tests for the serologic diagnosis of typhus fever among Japanese Nationals were again carried out over the year at the request of Public Health and Welfare Section, SCAP. The technic used was that recommended by the Virus and Rickettsial Division, AMDR&GS. Specific rickettsial antigens were commercially prepared and supplied through the Army Medical Department Research and Graduate School. Soluble antigen prepared in this laboratory was included in the testing of all specimens but results were not reported. Where sera were positive with soluble antigen but negative with the specific epidemic and murine rickettsial antigens, specimens were reported as negative. In so far as the occurrence of typhus fever in Japan could be judged from serum specimens received for testing at this laboratory, both epidemic and murine typhus appeared in relatively small numbers of cases scattered generally throughout the main islands. Only in Osaka ken, and to a lesser extent in Kyoto ken, did epidemic typhus cases reach a volume which would indicate an unusual incidence of the disease. More than half of the epidemic positive cases were from this area, with the remaining number accounted for by isolated cases in 27 different prefectures. Murine typhus appeared to be spread thinly from Hokkaido to Kyushu with Gifu ken submitting the greatest number of positive specimens of any single prefecture (21 cases, or 22 percent of the murine positive group).

Only a small number of positive specimens of any type were received after the middle of June. Over the period of a year, 1,366 serum specimens representing 870 suspect cases were received for testing. In a number of instances only a single specimen per case was submitted of which some were found to be anticomplementary. Final results were compiled from complement fixation tests performed on suitable sera from 837 suspect cases. These are listed by prefecture in Table 1. On the basis of the sera tested, it was found that a little under half of the suspect cases proved to be serologically positive for typhus complement-fixing antibodies, although in some prefectures the proportion was considerably less. For example, sera from only 3 of 81 cases in Fukui-ken gave positive results; in Tokyo 32 of 125 cases were positive. On the other hand, 152 of 180 Osaka cases showed positive reactions.

The highest seasonal proportion of epidemic typhus positive sera was seen in specimens received in April through June; lowest in February and March (Table 2). Samples from one fifth of the positive cases tested during the years' period could not be differentiated by means of the complement-fixation reaction. Rickettsial agglutination tests carried out on a statistically insignificant number of these latter specimens showed that while the majority of the sera not differentiated by the complement-fixation test gave agglutination reactions indicative of the murine form of typhus, almost a third showed identical titers with both epidemic and murine antigens. Further work will almost surely point to the existence of a serologically intermediate form of typhus in Japan.

Table 1. Results of Typhus Fever Complement Fixation Tests With Sera from Japanese Nationals, 1 January to 31 December 1948

Prefecture	Number of Suspect Cases	[x] Number of Sera	Positive Epidemic	Positive Murine	Positive Type Undetermined	Negative
Aichi	38	60	3	7	9	19
Akita	5	6	2	0	0	3
Chiba	14	26	3	3	3	5
Fukui	81	95	2	0	1	78
Fukuoka	28	36	5	5	3	15
Fukushima	11	22	7	0	0	4
Gifu	54	110	5	21	7	21
Hiroshima	21	31	2	3	1	15
Hokkaido	9	9	1	3	0	5
Hyogo	10	13	3	0	0	7
Ibaragi	16	19	11	0	1	4
Iwate	9	14	7	0	0	2
Kagawa	5	5	0	3	0	2
Kanagawa	43	54	3	7	5	28
Kochi	1	2	0	0	0	1
Kumamoto	4	7	0	1	0	3
Kyoto	42	50	28	1	1	12
Mie	3	3	0	1	0	2
Miyagi	4	5	2	0	0	2
Miyazaki	2	3	0	0	0	2
Nagasaki	33	39	1	7	7	18
Nara	24	32	5	2	2	15
Okayama	3	4	0	2	0	1
Osaka	180	318	119	12	21	28
Saga	5	5	0	0	1	4
Saitama	25	37	4	2	4	15
Shimane	11	13	1	2	1	7
Shizuoka	7	9	0	1	1	5
Tokyo	125	174	12	11	9	93
Tottori	8	10	0	3	1	4
Toyama	2	4	0	0	1	1
Yamagata	2	2	0	0	0	2
Yamanashi	7	16	0	0	0	7
Unknown	5	5	2	0	1	2
Total	837	1238	228	97 (405)	80	432

[x] For this final compilation, all anticomplementary sera have been deleted from calculations. Only cases with suitable serum samples have been included.

The relatively great amount of purified rickettsial antigen required for the tube agglutination test has precluded the application of this useful diagnostic tool as a routine procedure at this laboratory. This has led to a very real loss in accuracy of the laboratory diagnosis of typhus fever and has contributed to an erroneous impression concerning the actual distribution of the disease. As stated earlier, sera showing positive complement-fixation with soluble antigen but negative reactions with specific rickettsial antigens (Breinl and Wilmington strains) were reported as negative. A limited number of tests have indicated that in a high proportion of cases, such sera show positive rickettsial agglutination reactions, usually of the murine specific type, but occasionally of the intermediate type. Tests have been on too small a scale to determine whether epidemic positive sera may also be encountered. Work is in progress to check the accuracy of a slide agglutination technique as compared with the conical tube method. If similar results are obtained, a far greater number of sera can be tested quantitatively than is possible with the present method.

Table 2. Typhus Complement Fixation Results : Monthly Tests for 1948

Period	Positive Cases	Complement Fixation: Positive Epidemic	Complement Fixation: Positive Murine	Complement Fixation: Positive type Undetermined
1 Jan. - 31 Jan.	x 73/133 - 55%	37/73 - 50%	18/73 - 25%	18/73 - 25%
1 Feb. - 29 Feb.	39/95 - 41%	5/39 - 13%	21/39 - 54%	13/39 - 33%
1 Mar. - 31 Mar.	45/86 - 52%	12/45 - 27%	25/45 - 56%	8/45 - 18%
1 Apr. - 31 May	155/312 - 50%	120/155 - 78%	15/155 - 10%	20/155 - 12%
1 Jun. - 30 Jun.	45/87 - 52%	32/45 - 71%	8/45 - 18%	5/45 - 11%
1 Jul. - 31 Jul.	9/24 - 38%	5/9 - 56%	1/9 - 11%	3/9 - 33%
1 Aug. - 30 Nov.	11/32 - 34%	5/11 - 46%	3/11 - 27%	3/11 - 27%
1 Dec. - 31 Dec.	28/68 - 41%	12/28 - 43%	6/28 - 21%	10/28 - 36%
1 Jan. - 31 Dec.	405/837 - 49%	228/405 - 56%	97/405 - 24%	80/405 - 20%

x Numerator represents number of cases positive
Denominator represents number of suspect cases

In addition to the sera tested from Japanese Nationals, a small number of specimens was received from Korean cases early in 1948. All positive cases (21/31) were of the epidemic type.

Special

A New Endemic Area of Tsutsugamushi Disease in Japan - While references to the disease now known as tsutsugamushi disease or scrub typhus have been found in Chinese writings of the 16th Century, first careful descriptions of the infection as a clinical entity appeared in reports of Japanese investigators and physicians. The epidemiology and etiologic relationship of the mite vector were worked out in many of their principal features several decades before the causative rickettsiae were known. Tsutsugamushi disease in Japan soon came to be associated with grassy flood lands in fertile river valleys in limited areas of three prefectures of northwest Honshu (Agano and Shinano Rivers, Niigata Ken; Mogami River in Yamagata Ken; Omono River in Akita Ken; and along the tributaries of these rivers). Occurrence of the disease was found to be sharply seasonal with a large majority of the cases being reported in July, August and September. Case mortality was reported to range from 20 to 60 percent or more.

Later, Japanese workers showed that tsutsugamushi disease occurred not only in Japan but also in Formosa under a variety of ecological conditions including those found on plantations in forests, at the foot of mountains, and at elevations up to 6,500 feet. In the Pescadores (Boko) Islands, the disease was found to occur in the vicinity of dwellings near coral walls harboring various species of rats and their vector trombiculid mites. Tsutsugamushi disease in these latitudes was found to have a considerably longer seasonal incidence and a lower case fatality rate.

Gradually it became apparent through the work of a small group of Dutch, Australian and English investigators that the "pseudo typhoid" or mite fever of Sumatra and the "K form of tropical typhus" or "rural typhus" in the Federated Malay States, occurring at the edge of jungles on estates or plantations covered with brush and coarse grasses, closely resembled tsutsugamushi disease. Although the Dutch in particular maintained for a time that the tropical forms of the disease were not identical with the classical tsutsugamushi disease, it came to be generally accepted that both were caused by related strains of Rickettsia orientalis. Similarly, the "coastal fever" of the scrub lands in North Queensland, "endemic typhus" of the jungles and swamps in mandated Territory of New Guinea, "tropical" or "scrub" typhus in flood lands of Indo-China, and the rural mite typhus of India were all recognized to share a common etiology with tsutsugamushi disease prior to the outbreak of the war with Japan.

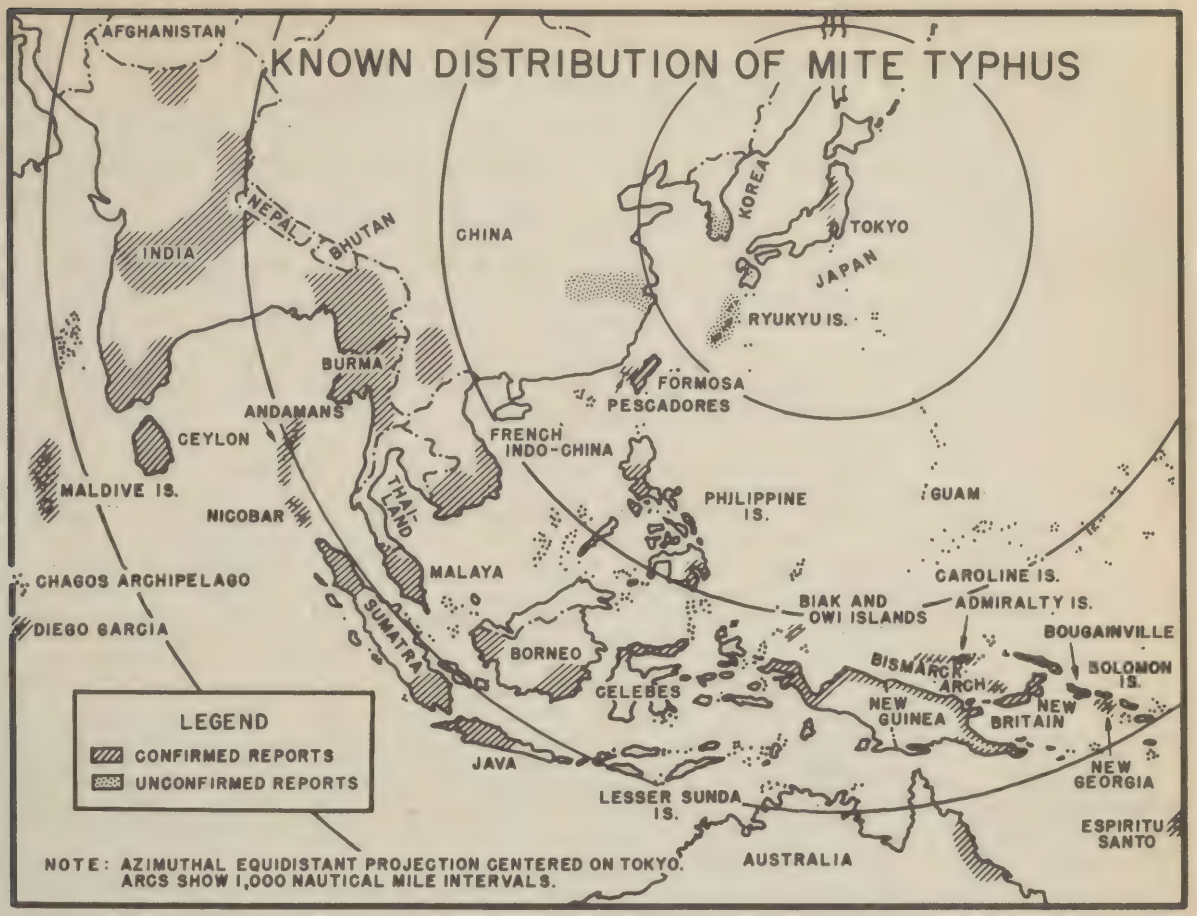


FIG. 1

Following onset of hostilities and the consequent large scale military operations throughout the southwest Pacific and the China-Burma-India Theater, the disease was encountered in widespread areas throughout southeast Asia and the southwest Pacific in all seasons and under a large variety of climatic and ecologic conditions. The known geographical distribution of tsutsugamushi disease at the time of cessation of military operations is represented in Figure 1. Included in the sketch is a new area in Honshu to be described in this report.

1. Occurrence of Cases in Military Personnel - The possibility that tsutsugamushi disease might occur in localities in Japan other than the known highly endemic areas of northwest Honshu mentioned previously was raised by a report of several suspect cases of tsutsugamushi disease in military personnel. Diagnosis was made on clinical grounds at the 35th Medical Station Hospital, Kyoto. Upon investigation it was found that three young soldiers showed in varying degree all the typical signs and symptoms of a mild form of the disease. Single or multiple eschars, regional lymphadenopathy, generalized macular rash, pharyngitis, conjunctivitis, slight nuchal rigidity, frontal headache, spiking temperature, and leukopenia were all noted. There appeared to be little doubt concerning the accuracy of the diagnoses which were later confirmed by laboratory studies.

All of the original patients were members of Battery A, 8th Field Artillery Battalion, 25th Division Artillery, who had returned on 19 October 1948 from training in the Fujino-Susono Maneuver Area at the southeast base of Mount Fuji near Gotemba Machi, Shizuoka Ken. (See Figure 2). Members of the Battery had been in the Maneuver Area from 3 September to 19 October when they returned to Nara. On two nights (exact dates not known) during the last two weeks of their training period, the troops had slept on the ground in a brushy area at the side of a small ravine. The first case developed symptoms of disease on 19 October, followed by cases on 20 and 22 October, respectively.

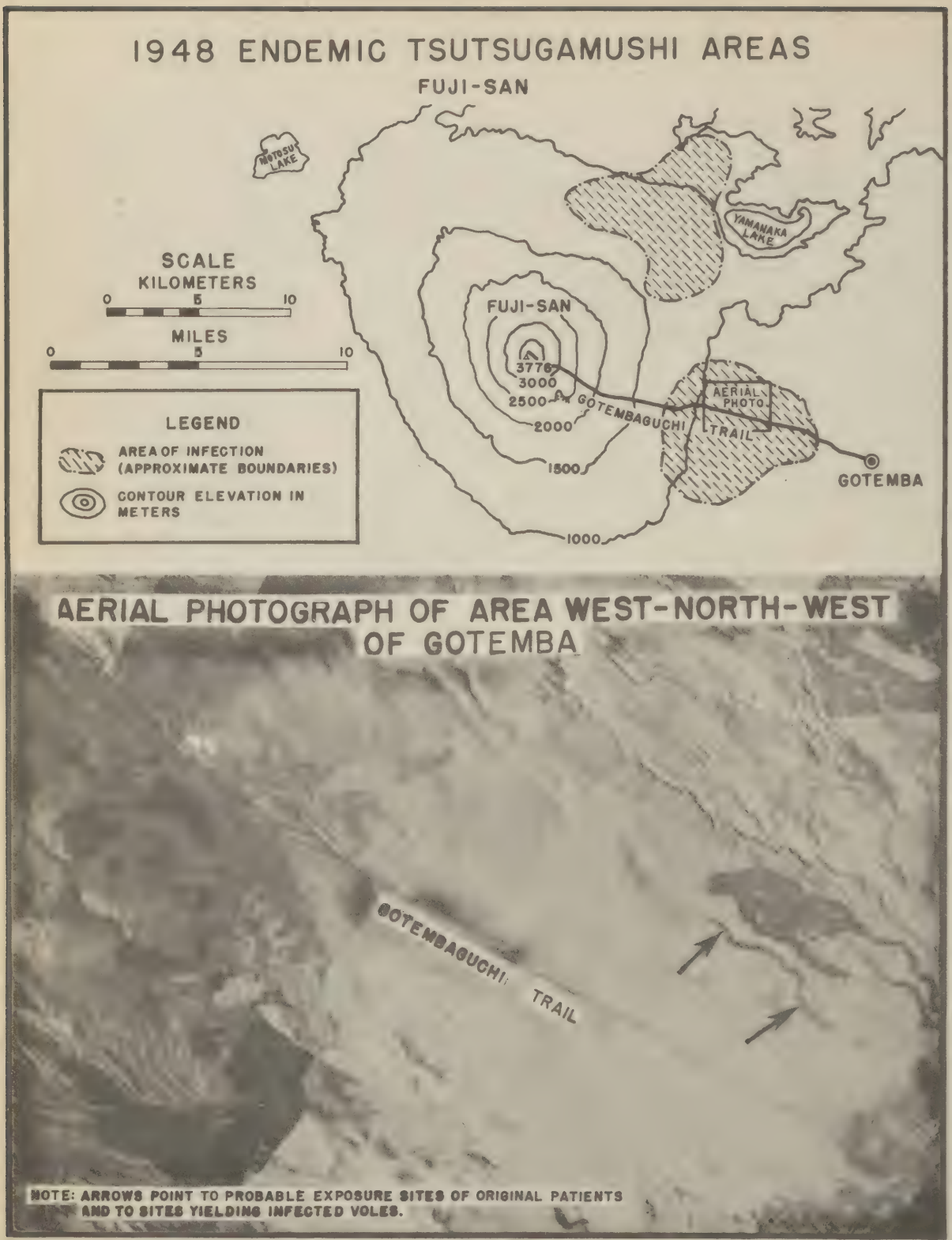

1948 ENDEMIC TSUTSUGAMUSHI AREAS
FUJI-SAN
MOTOSU LAKE
YAMANAKA LAKE
SCALE
KILOMETERS
0 5 10
MILES
0 5 10
LEGEND
AREA OF INFECTION (APPROXIMATE BOUNDARIES)
CONTOUR ELEVATION IN METERS
FUJI-SAN
3776
3000
2500
2000
1500
1000
GOTEMBAGUCHI TRAIL
AERIAL PHOTO
GOTEMBA
AERIAL PHOTOGRAPH OF AREA WEST-NORTH-WEST OF GOTEMBA
GOTEMBAGUCHI TRAIL
NOTE: ARROWS POINT TO PROBABLE EXPOSURE SITES OF ORIGINAL PATIENTS AND TO SITES YIELDING INFECTED VOLES.

FIG. 2

Questioning of the patients elicited the story that at least one other of their companions had shown a fleeting rash and complained of headache a few days previously but had not reported on sick call. An examination of the remaining enlisted personnel of A Battery was carried out at Nara in an attempt to uncover missed cases of the disease. In approximately 15 of 80 individuals, inguinal or axillary lymphadenopathy of unexplained cause and/or lesions suggestive of eschars were noted. Three of the group of 15 showed faint macular rash, and from these blood samples were obtained for further study. All individuals claimed to be free of any subjective symptoms of disease such as headache or malaise. Specimens from the 3 individuals bled proved to be negative for Proteus OXK agglutination, but follow-up specimens drawn 11 days later showed titers of 1:640, 1:160 and 1:80, respectively. From one of the 3 (Yoxthiemer), mice inoculated with ground blood clot from the original specimen have apparently yielded a strain of tsutsugamushi disease now in fourth passage. One individual (Jones) of the suspect group showed marked inguinal lymphadenopathy and complained of malaise. An ulcerated lesion was found on the inner thigh. This subject later developed a typical case of the disease.

Following diagnosis of tsutsugamushi disease in the 35th Medical Station Hospital and dissemination of this information, other cases were diagnosed and confirmed serologically among military personnel of several additional units which had also engaged in maneuvers in the Gotemba area. A single known case later occurred in an officer from Camp McNair, a few miles northwest of Gotemba.

2. Epidemiological Features - Between the first of August and the last of October, 1948 between 17 and 18 hundred troops took part in maneuvers held in the 25th Infantry Division Maneuver Area in Fuji Region near Gotemba. The organizations participating were in the areas at different times during this period. Table III shows the various organizations concerned, their average strengths and the time they were in the area. The first two of the units listed, comprising 753 men of the 1769 total, left the area prior to 1 October. No cases of scrub typhus occurred in these organizations as far as known. Those groups which did experience the disease, remained well into October.

The area and some aspects of the terrain occupied by the maneuvering troops are shown in Figure 2. The area was generally hilly with soil for the most part, made up of porous, volcanic ash which was well drained. Except in camps and bivouac areas, grass, weeds, brush and scrub trees were abundant. While there is said to be no truly typical terrain where the infected vectors of the disease are found, in general semi-flat, rather poorly drained areas with considerable moisture in the soil, together with fairly dense grass, shrubbery etc., are considered to be the most likely sites. This area does not entirely fit this description. However, since moisture is considered to be a requirement, it is of interest to note that during the months of August and September 35.27 inches of precipitation was reported by the Gotemba weather station. This may be compared to 23.6 inches for the similar period in 1947. In fact, there was appreciably more rain during the entire summer season of 1948 (64.5 inches June to September), than for the same months in 1947 (37.3 inches). This may assume some significance when it is recalled that, during the months of July and August, 1947, between 1400 and 1500 men manoeuvered in the same area and no cases of scrub typhus were reported.

Table III. Troops In Fuji Maneuver Area 1948

	Dates	Organization	Strength (Average)
1.	5 Aug. - 3 Sept.	1st Bn 24th Inf Regt	707
2.	11 Sept. - 25 Sept.	CO F, 1st Bn 24th Inf Regt	46
3.	22 July - 31 Oct.	77 Combat Engr Co 24th Inf Regt	97
4.	4 Oct. - 24 Oct.	3rd Bn 24th Inf Regt	534
5.	3 Sept. - 19 Oct.	159 Field Arty Bn	273
6.	4 Sept. - 19 Oct.	A Bat 8th Field Arty Bn	112
	Totals		1769

The duties and activities of the men within the several units varied considerably. Some were engaged in active combat type operations necessitating close and relatively prolonged contact with the ground, grass and shrubs. Others performed supply, maintenance and camp garrison type duties requiring little actual contact with the ground and brush. The significance of these diverse activities will be

spoken of later. Most billeting was in tents or buildings, and cots were generally provided. Ground sleeping, with sleeping bags, was practiced by some units for a few nights only.

The first case, subsequently confirmed as scrub typhus, was admitted to the hospital on 19 October 1948, following symptoms of about 12 hours duration. Following this, 23 cases were admitted between 22 October and 17 November 1948 - all but three of these to the 35th Station Hospital. With the generally accepted incubation period being between one and three weeks, this places exposure in October. This apparently is confirmed by the facts previously stated that the organizations leaving the area prior to 1 October did not experience the infection.

The distribution of the cases among the various organizations and units is shown in Table IV. It is seen that the overall attack rate was just over 2 percent while the unit attack rates varied between one and six percent with even wider spread among the sections within the units.

Table IV. Occurrence of Cases by Units

	Unit	Strength (Average)	Clinical Cases	Attack Rate
I.	3rd Bn 24th Inf Regt			
	Hq & Hq Co	49	-	-
	I Co	122	6	4.9%
	K Co	129	3	2.3
	L Co	137	2	1.5
	M Co	97	-	
	Total	534	11	2.1%
II.	77th Combat Engineers	97	1	1.0%
III.	159th Field Artry Bn			
	Hq Batry	68	-	-
	A Batry	79	2	2.5%
	B Batry	85	3	3.5
	Sv Batry	31	-	-
	Med Det	10	-	-
	Total	273	5	1.8%
IV.	A Batry			
	8th Fld Batry Bn			
	Survey Grp	10	-	-
	Communication Section	25	4	16.0%
	Firing Batry	55	3	5.5
	Mess and Maint	22	-	-
	Total	112	7	6.3%
	Grand Total	1016	24	2.4%

It has been possible to associate, to an apparently significant degree, the activities of the units and actions with the extent of occurrence of the disease. The 77th Combat Engineers, although in the area longest, had the lowest attack rate. This organization was engaged in maintenance and repair work and had relatively little intimate contact with the ground and brush. The Headquarters and Headquarters Company Service Battalions, mess and maintenance groups within the units experienced no cases. Rather, it was the personnel of the firing batteries and other sections whose activities required close and prolonged contact with the soil, grass and shrubbery, who contracted the infection. For example, of the 25 individuals in a communication section of a battery of the 8th Field Artillery Battalion, four developed clinically recognizable cases of scrub typhus. Subsequently, it was determined that this section had unusually heavy exposures to the ground and brush as compared to the other sections of the battery. Also, the only cases developing in the 159 Field Artillery Battalion were among personnel in the Firing Batteries. The activities of these organizations included the digging of pits for gun emplacements, and the cutting and handling of grass and shrubbery for use as camouflage.

Similarly, with the exception of Company M, it was the actively maneuvering units of the Third Battalion of the 24th Infantry Regiment which were affected. Of these, Company I had a considerably higher attack rate than the other. (This unit trained in area not previously used by other organizations during the season). There is no apparent reason why the personnel of Company M escaped the disease, since their activities were the same as those of the other three companies in which infections occurred. However, since the attack rate for the entire organization was in the neighborhood of 2 percent, chance variation alone might well explain the absence of cases.

In addition to the cases of scrub typhus occurring in the 25th Division Maneuver Area, one case was reported from the First Squadron, 5th Cavalry Regiment which was at Camp McNair from 11 July to 4 November. This case occurred in an officer who was unusually energetic in his field activities, covering most of the area on foot, sleeping out on occasion and, in general, subjecting himself to considerable potential exposure. An officer who frequently accompanied him was found by laboratory studies to have had an inapparent infection.

The cases discussed were those which were clinically recognizable. These do not represent the extent of infection with the scrub typhus organism; however, their distribution throughout the organizations is such that they would appear to serve as a reasonably accurate index of the manner and extent of the occurrence of infection.

(Note: This discussion of epidemiology, reproduced here to provide continuity, was prepared by Lt. Colonel A. P. Long, MC, Office of the Surgeon, FEC, on the basis of information obtained from various sources.)

3. Inapparent Infections - Following the demonstration of clinical cases random selected blood samples were obtained on various members of units reporting cases. On the basis of serial OXK reactions at least an equal number of extremely mild or inapparent infections occurred. Since relatively few "normals" were examined it is not possible to estimate the magnitude of such infections. Further substantiation of the occurrence of mild cases is given by the apparent isolation of a rickettsial strain from an individual with no subjective symptoms (discussed under Isolation Studies below).

4. Field Investigations - Epidemiological considerations pointed clearly to the fact that the present series of cases of tsutsugamushi disease arose from exposure to vectors of the disease in the military maneuver areas at the foot of Mount Fuji, both in the Gotemba and in the Camp McNair localities. Field trips were made in late October and mid-November to these areas in a search for probable rodent hosts and vector mites. Although none of the original group of patients in Kyoto or the other members of Battery A at Nara had seen either chiggers or ticks or were aware of bites of these insects, the presence of typical eschars on personnel just returned from the Gotemba area, at least, made it seem extremely likely that mites could be found.

In the 25th Division Maneuver Area, which was the first investigated, it was decided for purposes of a preliminary investigation to confine attention to camp site areas where troops had slept on the ground. These sites were located in brushy terrain at the edge of a small ravine shown on the aerial photograph (indicated by arrows in Figure 2). Scrub growth of trees, bushes and coarse grasses abounded throughout the area. Soil consisted of porous black volcanic ash. Numerous evidences of small burrowing rodents were seen in the camp site areas and along the banks of the ravine. Grasses resembling the "susuki" grass (Miscanthus sinensis) were relatively common. (This tall grass is found extensively in infected areas of Niigata and Yamagata prefectures).

Trap lines were set in likely places, and a high percentage of catches made using bacon rind and dried cereal grains as bait. In the area examined, no Microtus montebelli voles, known to act as the principal "reservoir" host of tsutsugamushi disease of northwest Honshu, were captured. Far eastern field mice (Apodemus speciosus speciosus Temmink) and northern mole shrews (Urotrichus talpoides hondonis Thomas) were present in considerable numbers, and both species yielded numerous mites as well as lice and a few fleas. Splenic and liver tissue suspensions from each species were pooled and inoculated into white mice for attempted recovery of rickettsiae. Pools of mites recovered from the rodents were also inoculated.

A spot survey was also made in the Camp McNair area for rodents and mites. General topography was similar to that seen in the Fujino-Susano area with rolling hills, wooded areas, scrub areas, and open spaces covered with low grasses. Top soil was composed of weathered lava ash varying from loose porous material to rough lava blocks. Rodent surveys were made in four small localities. Rodents appeared to be most abundant in a previously cultivated field overgrown with low grasses, and in a small forested

area. As at the Gotemba area, the predominant small rodent species, as far as could be determined by trapping, appeared to be Apodemus speciosus. Northern mole shrews were less numerous than in the 25th Division area. The only two field voles (Microtus montebelli) captured in either survey were recovered on D-range in a small brushy patch within 100 feet of firing bunkers which had been used on the maneuvers. As before, both pooled mites and rodent tissues were inoculated intraperitoneally into white mice for infectivity studies.

5. Recovery of Rickettsiae: a. Human cases - Because of the pressure of other work, attempts to isolate Rickettsia orientalis from human patients were discontinued after a few positive results were obtained. In no cases have cross-immunity studies been completed for final identification, and isolations here reported have been termed strains of R. orientalis on the basis of the clinical picture in human cases, the finding of typical intracellular rickettsiae in smears from peritoneal exudate in passage mice, together with the characteristic gross pathologic appearance (peritoneal and pleural sticky exudate, lymphadenopathy, spleen enlargement) seen in mice showing symptoms of the disease.

In four cases strain establishment is unquestionable. Rickettsiae are found consistently, and mice have died with regularity since the third passage on the 9th to 12th day after inoculation with all the typical symptoms of the disease. These include:

1. Jones, Charles, Sgt. ASN 17204020, Battery A, 8th Field Artillery Battalion. Strain isolated from blood clot 28 October 1948; temporarily designated Gotemba 2. Infection occurred in Fujino-Susono Maneuver Area. (This individual found in physical inspection on 25 October to show ulcerated eschar and prodromal symptoms of disease). OXK titers: 28 October 1:80; 1 November 1:80; 4 November 1:40; 8 November 1:160; 13 November 1:80; 20 November 1:640; 26 November 1:80; 10 December 1:40.

2. Caviness, Joseph, Pvt., RA 13241537, 3rd Bn, Co. K, 24th Inf. Regt. Strain isolated from blood clot 1 November 1948; temporarily designated Gotemba 3. Infection occurred in Fujino-Susono area. Admitted to 395th Station Hospital 29 October 1948. OXK titers: 29 October negative; 1 November 1:80; 8 November negative; 17 November 1:320; 22 November 1:2560; 29 November 1:640; 6 December 1:320; 13 December 1:160.

3. Hinnant, Ned, 3rd Bn. Co. I, 24th Inf. Regt. Strain isolated from blood clot 9 November 1948. Infection occurred in Fujino-Susono area. Admitted to 35th Station Hospital 2 November 1948. OXK titers: 4 November 1:20; 9 November 1:160; 13 November 1:640; 19 November 1:1280; 26 November 1:640; 10 December 1:160.

4. Shea, Leonard, Lt. Colonel, 1st Cavalry Division. Strain isolated from blood clot 15 November 1948. Infection occurred in Camp McNair area. Admitted to 49th General Hospital 13 November 1948. OXK titers: 13 November negative; 22 November 1:640. (This is the only case of tsutsugamushi disease known to have occurred in the Camp McNair area).

In three cases the gross pathological picture in passage mice is entirely compatible with experimental tsutsugamushi disease but rickettsiae have not been found regularly and only occasional mice have died through the 4th passage. These include:

1. Yoxthiemer, Herbert, Rct. ASN 13217012, Battery A, 8th FA Bn. Passage initiated from blood clot of 25 October 1948. Probable infection incurred in Fujino-Susono area. This individual has shown no subjective symptoms of disease at any time. Bled for inoculation because of faint macular rash (said by subject to be "sweat" rash) and axillary lymphadenopathy at time of physical inspection. OXK titers: 25 October negative; 5 November 1:160; 11 November 1:80; 15 November 1:40.

2. Cope, Robert, Rct., Battery A, 8th FA Bn. Onset 22 October 1948. Passage initiated from blood clot of 25 October 1948. Infection occurred in Fujino-Susono area. OXK titers: 25 October negative; 27 October 1:20; 2 November 1:160; 8 November 1:640; 13 November 1:160; 19 November 1:320; 26 November 1:320.

3. Napier, Lee, Pvt., Battery A, 8th FA Bn. Onset 21 October 1948. Passage initiated from blood clot of 25 October 1948. Infection occurred in Fujino-Susono area. OXK titers: 23 October negative; 26 October negative; 30 October 1:80; 9 November 1:160; 15 November 1:160; 22 November 1:320; 26 November 1:80; 10 December 1:40.

In two additional cases, passage mice have shown no deaths through the third passage, but sacrificed mice have shown scanty peritoneal exudate, slight spleen enlargement, and knotting of lymph nodes. These include:

1. Claycomb, William, Pvt., RA 19302758, 3rd Bn., Co. I, 24th Inf. Regt. Admitted to 395th Station Hospital 26 October 1948. Infection occurred in Fujino-Susono area. Passage initiated from clotted blood 1 November 1948. OXK titers: 27 October 1:320; 1 November negative; 8 November negative; 17 November 1:20; 22 November 1:80; 30 November 1:160; 6 December 1:40; 13 December 1:20.

2. Stevenson, James E., Pvt., RA 18301925. Admitted to 395th Station Hospital. History not available. Passage initiated 1 November 1948. OXK titer: 2 November negative; 10 December 1:80.

b. Hosts and Vectors - Of the various spleen and liver pools from shrews, field mice and voles inoculated into white mice, only two show possibility of rickettsial recovery in the third passage. Passage mice from one mole shrew tissue and from one Apodemus speciosus tissue pool have shown slight spleen enlargement and peritoneal exudate suggestive of infection with tsutsugamushi disease. Rickettsiae have not yet been observed in smear preparations. Passages from other tissue pools and from mite pools have shown negative results to date. Further blind transfers are being made.

6. Identification of Mites - While examination of mites collected from rodents in the Maneuver areas has not been completed, tentative identifications have been made on a number of specimens. Some host variations in mite attachments has been noted. Of the preserved specimens studies thus far, none of the trombiculid mites have been found to be the principal known vector for Japan,- Trombicula akamushi. This is not surprising in view of the fact that this species is found only rarely after September and collections were made in late October and mid-November.

Of 9 trombiculid mites from Apodemus speciosus (Gotemba area, October), all were found to be of a species previously unclassified. Detailed description is being prepared. Distinguishing characteristics are: scutum with posterolateral angles rounded, hind margin bowed; sensillary bases (SB) in rear of posterolateral (PL) line; dorsal setae (DS) total about 30 (DS1-2). No trombiculid mites have been found thus far in specimens taken from mole shrews (Gotemba, October). All belong apparently to the laelaptid group.

T. palpalis, T. scutellaris and T. japonicum (tentative identification) species have been identified among trombiculids from field mice taken in the Camp McNair area; only T. scutellaris species from Microtus montebelli. Final identifications will be reported later.

7. Previous Report of Case in Gotemba Area - Local physicians, and local and prefectural health workers in nearby villages and towns were questioned closely concerning possible tsutsugamushi disease in Gotemba or the surrounding territory. While some of them claimed to be familiar with the disease through previous experience in Formosa or Niigata ken, none of them was aware of any case known to have occurred or been suspected in regions other than northwest Honshu. A search of Japanese literature uncovered a single case report which has apparently received little or no reading even among Japanese investigators of the disease. In 1934, Heiji Sakita made a short report in the Japanese Army Medical Journal (Gunidan Zasshi 248:289) of suspected tsutsugamushi disease in a Japanese field artillery soldier from the Fuji Maneuver Area. The case was said to be mild and was diagnosed originally as "sepsis" so that no attempt was made to uncover the causative organism.

Recovery of Rickettsiae from Voles and Trombicula palpalis Mites - The tsutsugamushi disease vector group as defined by Wharton (Proc. Entomol. Soc. Washington 48: 171-178, 1946) includes only a few closely related species of the many species of trombiculid mites which have been described. As defined by Wharton, these vectors include Trombicula akamushi (Brumpt 1910), T. pallida Nagayo et al. 1919, T. scutellaris Nagayo et al. 1919, T. intermedia Nagayo 1920, T. deliensis Walch 1922, T. fletcheri Womersley and Heaslip 1933, T. obscura Womersley 1944, and T. fulleri Ewing 1945. Some questions exist as to whether or not T. deliensis is a separate species from T. akamushi, a subspecies, or a variant.

While in addition to T. akamushi, T. pallida, T. intermedia (Nagayo et al. 1921) T. fletcheri (Blake et al, 1945), and T. deliensis (Philip, 1945) have been shown to be infected with scrub typhus rickettsiae, T. palpalis has never been proved to be a carrier of the organism. Since Kawamura (1920) records the occasional parasitism of this species of mite on man, it is still a possibility, in spite of the fact that the species morphologically falls outside of Wharton's vector group, that a few cases of tsutsugamushi disease during the early and late months of the season may be caused by T. palpalis.

In April 1946 a strain was recovered from a pool of mites consisting largely of the latter species, although the presence of a small number of T. pallida (2 of 30) in the sample lot saved for identification made the source of the rickettsiae doubtful. It may be mentioned here that previous strain isolations from trombiculid mites, so far as can be determined from available literature, have all been made in a similar fashion, inoculating pools and saving samples for later identification.

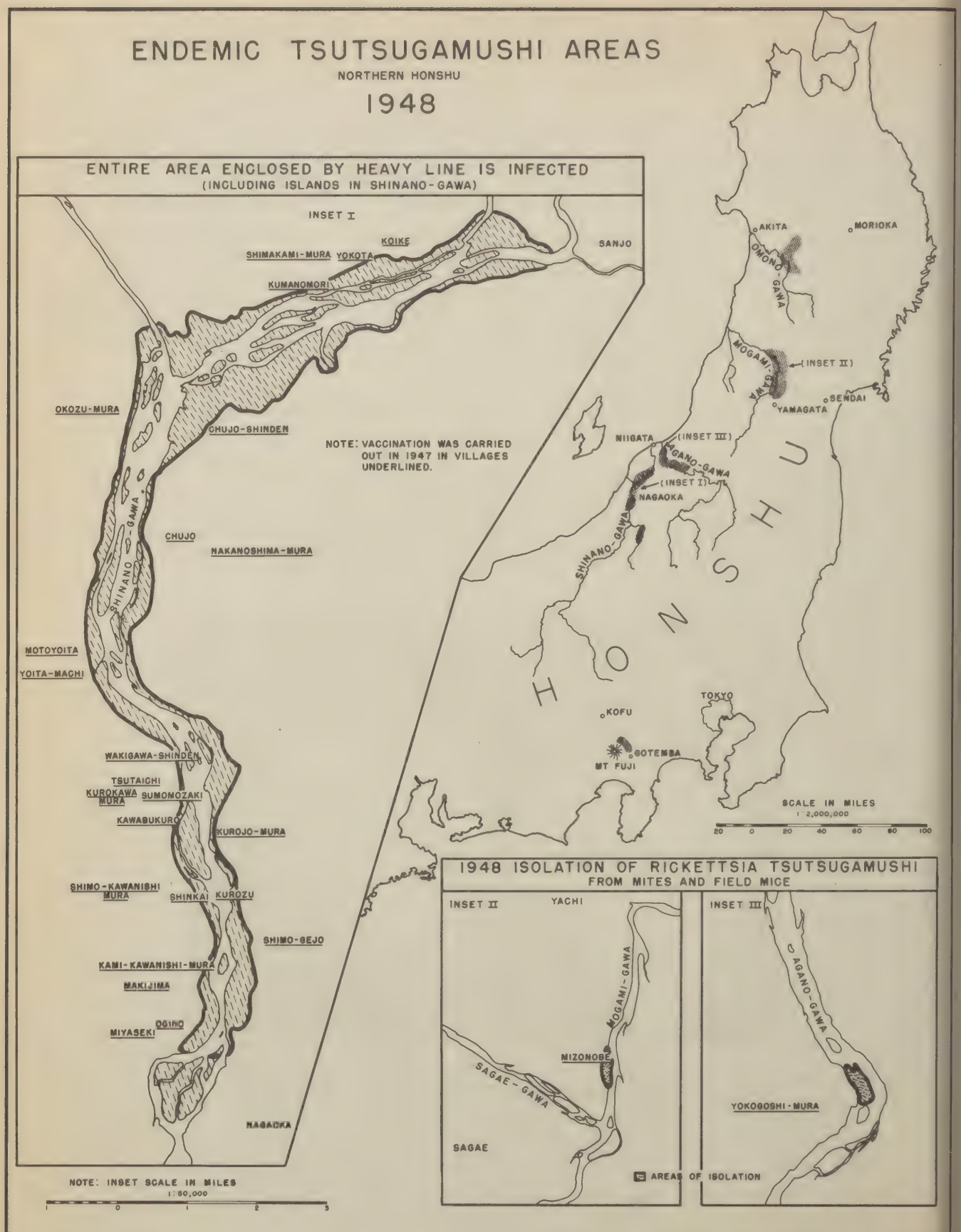

ENDEMIC TSUTSUGAMUSHI AREAS
NORTHERN HONSHU
1948
ENTIRE AREA ENCLOSED BY HEAVY LINE IS INFECTED
(INCLUDING ISLANDS IN SHINANO-GAWA)
INSET I
KOIKE
SHIMAKAMI-MURA
YOKOTA
SANJO
KUMANOMORI
OKOZU-MURA
CHUJO-SHINDEN
NOTE: VACCINATION WAS CARRIED OUT IN 1947 IN VILLAGES UNDERLINED.
SHINANO-GAWA
CHUJO
NAKANOSHIMA-MURA
MOTOYOITA
YOITA-MACHI
WAKIGAWA-SHINDEN
TSUTAICHI
KUROKAWA MURA
SUMOMOZAKI
KAWABUKURO
KUROJO-MURA
SHIMO-KAWANISHI MURA
SHINKAI
KUROZU
SHIMO-SEJO
KAMI-KAWANISHI-MURA
MAKIJIMA
OGINO
MIYASEKI
NAGAOKA
NOTE: INSET SCALE IN MILES
1:50,000
0 1 2 3
AKITA
MORIOKA
OMONO-GAWA
MOGAMI-GAWA
(INSET II)
SENDAI
YAMAGATA
NIIGATA
(INSET III)
AGANO-GAWA
(INSET I)
NAGAOKA
SHINANO-GAWA
HONSHU
KOFU
TOKYO
GOTEMBA
MT FUJI
SCALE IN MILES
1:2,000,000
20 0 20 40 60 80 100
1948 ISOLATION OF RICKETTSIA TSUTSUGAMUSHI
FROM MITES AND FIELD MICE
INSET II
YACHI
MOGAMI-GAWA
MIZONOBE
SAGAE-GAWA
SAGAE
INSET III
AGANO-GAWA
YOKOGOSHI-MURA
AREAS OF ISOLATION

FIG. 3

In March 1948, field voles (Microtus montebelli) and their attached mites were taken both in Yamagata Ken on the banks of the Mogami river and from the same Shinano river island near Yokogoshi, Niigata Ken, where the 1946 collection was made (Fig. 3). Both voles and mites were frozen in dry ice containers and transported to this laboratory. Upon return to Tokyo, voles were thawed and splenic tissue pooled and inoculated into white mice.

Mites were identified singly on a chilled stage under high power magnification. As was expected from the cold weather collection, no T. akamushi were found, T. palpalis and T. pallida were the only trombiculid mites present (ratio of 31 to 9 in Niigata and 58 to 3 in Yamagata). Other mite species, as identified by Dr. Baker of the U. S. National Museum, were Laelaps Kochi Oudemans and Atricholaelaps glasgowi (Ewing). T. palpalis mites were pooled separately and inoculated for rickettsial recovery.

No signs of infection were found through the fourth passage in mice inoculated with spleen pools from voles captured in Yokogoshi. In T. palpalis pools from both areas and in the spleen pool from Mizonobe (Yamagata ken), first passage inoculated mice showed slight enlargement of spleens and inguinal nodes. In the second and third passages, sticky peritoneal exudate began to appear, with rickettsiae first seen by smear in the third and fourth passages. Mortality has remained low. By the 6th passage about a 50 percent mortality was reached with deaths occurring from 17 to 30 days after inoculation. From the 4th passage on, all 3 strains have been characterized by production of large amounts of ascites fluid (10-15 cc.) in inoculated mice (see Fig. 4).

Immunity tests to determine relationship of vole and T. palpalis strains to known strains of human origin have not been completed. Initial tests have shown that recovered mice are susceptible to challenge with 10^{-1} and 10^{-2} dilution of highly lethal Agano X strain, but since these doses contain from 100,000 to 10,000,000 MLD_{50}, tests have been considered inconclusive. Mice recovered following inoculation with the T. palpalis strain from Niigata and challenged with Agano X in graded dilutions have been shown to be protected against 80 - 100 MLD of this agent. Mice recovered from inoculation with the Yamagata palpalis strain were protected in a single experiment against 200 MLD of the human strain. Using mice following recovery from inoculation with Shinano 11 and Shinano 13 human strains and challenging with the Niigata palpalis strain in 10^{-1} dilution, complete immunity was found. With Shinano 11 mice, none of 10 died following inoculation with a preparation which killed 4 of 10 control mice. With Shinano 13 mice, none of 10 in the immune group and 8 of 10 in the control group died after challenge with palpalis rickettsiae (Agano 13).

Male mice showing massive peritoneal exudate 15 days after inoculation with Agano 13 (Mite strain of rickettsia, probably R. tsutsugamushi)

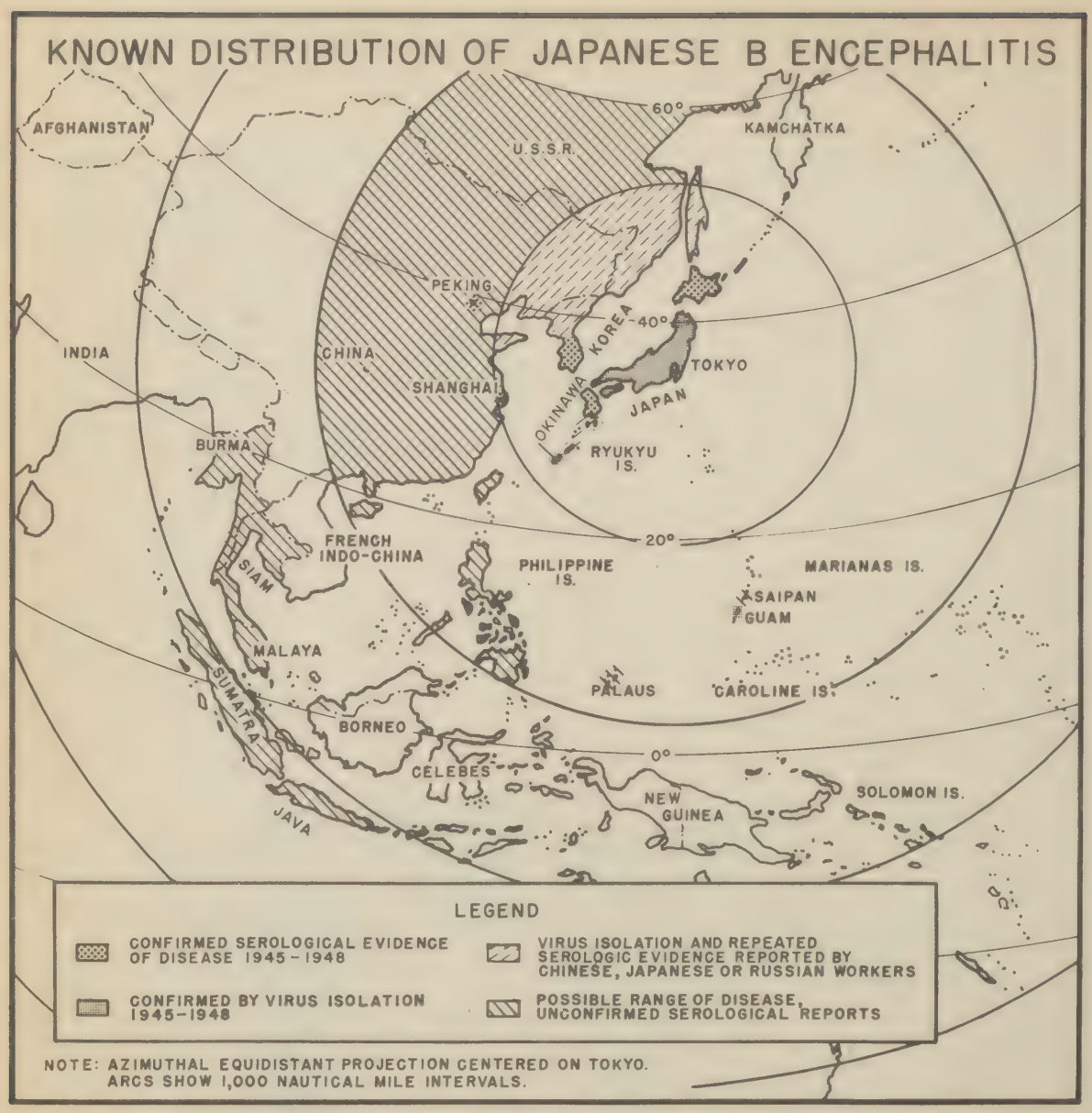


FIG. I

STUDIES ON JAPANESE B ENCEPHALITIS

During the past four years much interest has been shown by American workers in the various aspects of Japanese B encephalitis. This communication purposes to provide a cohesive background for a series of studies to follow. Emphasis has been placed on methods of transmission and prevention, including vaccination, but, of necessity, the study has expanded to all phases of the disease.

I. Introduction

Summer encephalitis in epidemic form is thought to have occurred in Japan as early as 1871, although the clinical pictures are difficult to differentiate from epidemic meningitis and poliomyelitis (1). Between 1919 and 1922 several hundred cases of encephalitis lethargica (Von Economo) were reported in

Japan as occurring without seasonal change in rates (1, 2). In July 1924 there was an outbreak of summer encephalitis of 6,125 cases with a mortality of 62%, creating panic among the populace. Almost 2,000 cases were reported in the prefecture of Kagawa (Shikoku), while 654 cases were reported in Okayama prefecture (Honsnu) (3). In 1928 Kaneko and Aoki (2) presented a comprehensive paper in which they concluded that the acute epidemic encephalitis occurring in Japan in the late summer and autumn was sufficiently distinct to warrant differentiation from encephalitis lethargica. Since both forms of encephalitis occurred in Japan these authors designated encephalitis lethargica as type "A" and the summer encephalitis as type "B".

The rather unwieldy term Japanese B encephalitis has since become familiar in the world's literature as characterizing a summer encephalitis of varied clinical aspects occurring especially on the main islands of Japan. Recent work has indicated that the disease also occurs in horses (4) and that it is frequently an encephalomyelitis. Since there is no definite method of naming the various diseases comprising the "arthropod-borne" encephalitides, other than that based on priority, the name Japanese B encephalitis has been retained in these studies.

Since 1924 the disease has continued to occur both in sporadic and epidemic form (3, 5). The two largest outbreaks were in 1935 with 5,370 cases and in 1948 with approximately 8,000 cases (6). Horse epizootics occur in Japan (4).

Literature on this disease is voluminous and no attempt will be made to summarize the reports at this time. English summaries are available in the Third Report on Epidemic Encephalitis by the Matheson Commission (7), in articles by Hammon (8) and by Warren (9), in a recent publication of the Surgeon, Far East Command (10), and in various standard text books on virus diseases (11, 12). The accuracy and completeness of these summaries suffer by reason of non-availability of many of the reports. Every effort has been made in this series of papers to authenticate each reference used, and citation of a reference does not necessarily indicate priority. Detailed reviews of certain aspects of the disease, not previously available in American literature are included when considered pertinent.

1. Etiology - Modern study of the disease began with the isolation of a virus from clinical cases by Hayashi during 1933 (13). He produced clinical disease in monkeys by the intracerebral injection of brain tissue of patients who had died of typical encephalitis and carried the virus for five passages. Following technics utilized by Webster (14) for the St. Louis encephalitis virus, Japanese workers (15) isolated numerous strains from patients dying in the 1935 epidemic. Characteristics of the strain were determined both in Japan (15) and elsewhere (16) and differentiation was made from related strains. A careful review in the original of articles published prior to 1934, indicates that the "isolations" made prior to this time probably are not related to the present strains of virus and most probably represented accidental contaminants or normally occurring infections in laboratory animals. Since none of these strains are available for study now, the practice (12) of ascribing properties to the virus listed in these earlier reports is confusing and should be discontinued.

(Note: The reference strain of virus usually used in Japan since 1940 is Kalanina, originally isolated at the Government Institute for Infectious Diseases during the 1935 Tokyo epidemic by Takaki et al (16a). Other known strains are stated to have been lost during the war years.

The "type" strain used in this laboratory is Nakayama. This strain was originally isolated by Kasahara, et al, (16b) in the Tokyo area in 1935-1936, the isolation being made from human spinal fluid by blind passage. Dr. Margaret Smith obtained this strain from Kasahara in 1938 (32). This was one of two strains studied by Sabin (54) and adopted for use in the production of the standard vaccine used in the studies to follow. At the same time the strain was registered at AMDR&GS (69) where it has been used as a reference since that time.

2. Serologic Aspects - Soon after the isolation of the virus it was noted that individuals recovering from the disease possessed antibodies in their blood serum capable of neutralizing or destroying the ability of virus suspensions to produce illness in laboratory animals (17, 18). These were termed virulicidins by the Japanese and are usually denoted neutralizing antibodies in American literature. In 1935 Kawamura (17) noted that not only serum from recently convalescent patients contained neutralizing antibodies, but that an individual who recovered from the disease four years previously also exhibited the presence of neutralizing antibodies. It was soon noted (19) that a relatively large proportion of the population of Japan and the domestic animals living in areas where the disease occurred either in endemic or epidemic form also possessed neutralizing antibodies. How long neutralizing antibodies persist is not definitely known but it appears to be several years in some instances (20).

In common with other neurotropic virus infections, patients suffering from Japanese B encephalitis usually develop complement fixing antibodies as well. American experience with complement-

fixing antibodies began when Hodes (22) used this method to determine the etiology of an outbreak of encephalitis on Okinawa, in 1945. There is little or no Japanese literature available on this phase of the serologic aspects of the disease. It now appears that complement-fixing antibodies are first noted between the fourth and tenth day of the disease, rapidly reaching a peak and declining in a matter of months. As is true of neutralizing antibodies, apparently normal individuals living in an endemic or epidemic area may also develop complement fixing antibodies but usually of a lower titer than that observed in clinical cases. A detailed discussion of the serology of Japanese B encephalitis will be presented later, and it is sufficient now to establish the fact that by serologic methods much information can be gained concerning diagnosis and the general epidemiology of the disease, that numerous inapparent cases occur during epidemic, and probably during non-epidemic years.

3. Geographic Distribution - Utilizing various combinations of serologic methods, as well as actual virus isolations (which are considered necessary for unequivocal proof of existence of the disease) many areas in the Far East, including the Pacific Islands, have been studied by workers of many nationalities. Figure 1 shows the presently established distribution. Distinction has been made as follows: (1) Areas in which virus isolation has been authenticated by American workers in the years 1945 - 1948 (23, 24, 25); (2) Areas in which there is confirmed serological evidence (obtained by American workers in 1945 - 1948) of the presence of the virus (25, 26, 27); (3) Areas in which virus isolation and/or repeated serologic evidence has been recorded by Chinese (28), Japanese (37, 30) or Russian (31) workers; (4) The possible range of the disease on the basis of unconfirmed serologic surveys (24, 32, 33, 34). Russian Autumn Encephalitis is considered, for purpose of this graphic presentation, as being identical with Japanese B encephalitis (31, 35).

It is essential to note that serologic "evidence" of the presence of the virus does not necessarily coincide with the occurrence of known clinical cases in humans or animals. Human cases are known to occur in Honshu, Shikoku, Kyushu, Hokkaido (25), Okinawa (23), Guam (24), Korea (Americans only (36)) and Eastern China (26). Cases are reported to occur anually in Formosa (37) and in Manchuria (30), as well as the Maritime district of Eastern Siberia (31). Confirmed occurrence of clinical disease in horses has been obtained only on the main islands of Japan, but is reported to occur in Manchuria (30).

4. Case Incidence - Japanese B encephalitis was not made a reportable disease in Japan until 1946 (38). Case incidence figures are generally considered to be reliable only during true epidemic seasons, and even then are probably high due to the incidence of similar clinical pictures such as brain tumors, apoplexy, etc., particularly in the elderly individuals. The diagnosis has usually been made on clinical grounds. (Confirmation can be had only by laboratory methods). Detailed statistical treatment of case incidence figures has been made by numerous workers in Japan, notably by Iimura (37) and Inada (5).

On the basis of reported figures prior to 1940 a prime characteristic of the disease appears to be its variability of occurrence. Attempts to explain this on an epidemiological basis have been legion and in general incomplete and unconfirmed. The status of this problem probably will not change until full information concerning the method of transmission is known. Outside of the Tokyo district the crude case distribution indicates a predominance in the young and the aged (37). When corrections are made for the age distribution of the population the actual percentage incidence in elderly people becomes much more pronounced. In the Tokyo epidemics of 1935 (5) and 1948 (39) crude case incidence showed a definite predominance in children below the age of 10 years. Detailed studies of the 1948 outbreak will form a later presentation.

Serological surveys (5, 24, 25, 27) in various areas of the Far East have shown an increasing percentage of individuals with positive neutralizing antibodies as the age of the surveyed groups increased, although this is influenced by the presence or absence of epidemics in years immediately preceding the surveys.

Because of the complicated and expensive laboratory procedures involved in the study of this disease it has been necessary to resort to relatively small samples of a population and per force to extend these figures so as to encompass an entire epidemiological group. It is thus necessary to tolerate certain possible fallacies and some of the apparently equivocal results may well be the function of small numbers. Further, there is definite evidence that marked distortion may be encountered if more than very limited geographical areas are treated as being homogenous.

5. Importance of Japanese B Encephalitis - From the standpoint of overall disease occurrence in Japan and other areas in the Far East, Japanese B encephalitis ranks well down in the list of major Public Health problems (41). As has been noted above, during the passage of years much of the population of Japan in certain areas has developed serologic evidence of experience with the disease and presumably a degree of immunity. This is in direct contrast to the adult population of the United States. When

war time activities resulted in the movement of a large group of individuals with no previous experience with the disease (and consequently no immunity) into areas where the virus is maintained from year to year and where periodic outbreaks of the disease occur, there was much speculation as to the possibility of extensive disease occurring in this non-immune, transplanted population. Further, there was no notion as to whether a population composed of non-immune adults would suffer the same proportion of inapparent cases as does a population containing many children and many partially immune adults. Consequently, investigation of the epidemiology of the disease and methods of preventing its occurrence in Americans became of prime interest to the military forces. Further, because of apparent similarities between Japanese B encephalitis and various other neurotropic virus diseases it is anticipated that any knowledge concerning the Japanese B encephalitis may be of significance in the investigation and prevention of any other disease in the group.

With the proof that a virus, serologically similar if not identical with the Nakayama strain of Japanese B encephalitis, is the causative agent of large scale horse epizootics (4) in Japan, an additional economic factor is apparent. Reports dealing with this aspect of the disease have been submitted previously, and a summation will be integrated in the present series.

6. Possible Methods of Control - Employing the well known principles utilized in the control of many disease infections for which there is no specific therapy or specific prophylactic drug, efforts early were made to protect by breaking the presumed cycle of transmission and to prevent the disease by vaccination. Much of the work since carried out on this disease and to be reported in the succeeding papers, has been devoted to these two aspects.

a. Reservoir and Vector Control - At the present time no definitive statement can be made concerning the possible reservoirs of this disease. Japanese investigators, notably the group headed by Mitamura (41), have advanced evidence to indicate that the true reservoir is the mosquito and that the virus can over-winter in the adult mosquito. This same group of workers (5) (and many others) have obtained evidence to indicate that the mosquito is responsible for transmission of the disease. Recently apparently undeniable American support for this possibility has been obtained (24). What other vectors may be involved is a fertile field for further study.

The influence of climatic factors on the incidence of disease presumably transmitted by mosquitoes is obvious. Long and detailed reports have been made by Japanese investigators. This data will be subjected to review in the light of experience gained during 1945 - 1948 and presented. The concensus of workers prior to 1945 was that the disease occurred usually in hot, dry summers but it is recognized that there were definitely other influencing factors.

Efforts at mosquito control have formed a large part of the measures adopted for the prevention of this disease. There is no good evidence yet to indicate that man-made efforts in this direction have been successful, nor is there evidence to deny the possible efficiency of this method of control.

b. Vaccination - Regardless of any success gained by measures against insect vectors, either as a result of individual protective devices or of area control, such measures are unlikely to be completely successful under semi-primitive housing conditions. Consequently, much effort and money has been expended in the attempt to prepare a potent vaccine as an aid in the prevention of this disease. Since a clinical infection with the virus appears usually to produce a lifetime immunity there is reason to believe that some success may be obtained by vaccination.

Japanese work on vaccine preparations began soon after the isolation in mice of the virus of Japanese B encephalitis. In 1938 Takenouti et al. (42) investigated a mouse brain vaccine inactivated by the photodynamic action of methylene blue; Mitamura et al. (19) observed the results of inactivation by tannic acid and combinations of heat and formalin on mouse brain vaccines; while Kaneko et al. (43) and Komiya et al. (44) reported their data on formalinized and heat-treated mouse brain vaccines. All of these experimental procedures were studied in mice, and suggested that a minimum, if any, immunity to an active virus challenge was produced by vaccination procedures.

Later Takenouti et al. (45, 46) re-investigated this problem with more encouraging results. Mouse brain vaccines inactivated by heat, phenol, ricinoleic acid, formalin or hematoporphyrin were prepared. The formalinized vaccine gave demonstrable immunity in mice and monkeys against a homologous challenge and produced neutralizing antibodies in the vaccinated animals. Takaki et al. (47, 48) prepared a formalinized mouse brain vaccine which gave immunity in mice based upon an active virus challenge. Kitayama, et al. (49, 50) prepared a lanolin ointment containing a mouse brain emulsion infected with the virus of Japanese encephalitis and applied this to the skin of rabbits resulting in a "remarkable production of neutralizing antibodies". Mitamura et al. (51) re-investigated the effect in mice of mouse

brain vaccines inactivated by heat, acetic acid, methylene blue, formalin, and immune serum and found the latter vaccines most effective.

These authors (51) and Kitayama et al. (50) studied the immune response in humans vaccinated with formalinized mouse brain vaccine and found that many such vaccinated individuals developed neutralizing antibodies.

Large scale vaccination of susceptibles in endemic areas of Siberia using a mouse brain vaccine has been advocated and practiced for the past five years (52). Smorodintseff has reported two cases among 10,485 vaccinated persons as compared to 59 cases in 8,030 non-vaccinated personnel. However, it is reported that Alperovich (53) could not confirm this work.

In the United States a formalinized mouse brain vaccine was developed by Sabin et al (54) and a chick embryo vaccine was produced by Warren and Hough (55), Koprowski and Cox (56) and Smadel et al. (57) and their immunogenic effect studied (58, 59, 60, 61, 62). Warren and Hough (55) observed that these two types of vaccines induced in mice a comparative resistance to infection with the homologous virus. Neutralizing antibodies were demonstrated in the sera of an equal proportion of human subjects following vaccination with either of the two types of vaccine (62).

Mouse brain vaccines possess certain potentially objectionable features, although very desirable immunological properties. As a prototype, vaccines containing brain tissue may on occasion produce a demyelinizing encephalopathy; chick embryo preparations appear to be free of this undesirable effect, but the fluid vaccines were not completely satisfactory because immunogenic potency decreased when stored at 5° C. for longer than one month. Therefore, a lyophilized chick embryo vaccine was developed by Smadel et al. (57). Assay values of all vaccines used in the following studies are based on standard NIH method of assay, determining the Minimal Immunizing dose in mice (63).

It has been necessary to consider the effect of age of the individual in evaluating vaccine response. Sabin (61) showed clearly in small groups that the response in children was much greater than in aged individuals. Presumably young adults should have an intermediate response (23) and data supporting this view will be reported later.

7. Vaccine Evaluation - The significant measures of the efficacy of a vaccine lies in comparison of disease occurrence (and/or severity of disease) in a vaccinated group and in a control group identical in all respects save vaccination. The comparison of non-identical groups may sometimes furnish auxiliary information. This type of study requires careful case evaluation, using serological confirmation (a rise in titer either measured by neutralization tests or complement-fixation in a patient with fever and varying neurological signs). A clinical diagnosis is sometimes the only available information if death ensues early in the illness.

Some of the succeeding papers will be devoted solely to the serologic response induced by vaccination. For purposes of this introduction it is sufficient to state that following administration of a potent (63) vaccine of killed virus various percentages of individuals can be expected to develop neutralizing antibodies. Such vaccination apparently does not prevent the subsequent occurrence of inapparent infections (as measured by complement-fixing antibody rise). Recent experience with chick embryo preparations indicates that vaccination alone, even repeated annual vaccination with dosage and type of vaccine now in general use, seldom induces complement-fixing antibodies in American adults, as measured by the methods described below. Occasional reactions at a 1:4 dilution are seen. In Japanese children repeated vaccination apparently induces an evanescent rise in complement-fixing antibodies if a sufficiently potent preparation is used. (The possibility of an anamnestic rise cannot be completely eliminated, but there is substantial evidence that previous infection does not enter into the picture).

Individuals immune because of previous experience with the live virus may not develop complement-fixing antibodies as a result of a new exposure to minimal quantities of the live virus such as might occur by natural methods of exposure. However, as first noted by Hammon (64) the administration of a potent vaccine to individuals, immune as a result of previous experience with the live virus, results in a prompt anamnestic complement fixation antibody rise. A probable explanation lies in a consideration of dose factors. (This response to a single dose of vaccine provides a very delicate tool for survey use). Detailed data to support the generalization of the last two paragraphs will be presented later but they are given at this time to permit orientation. Further work, including animal studies, is in progress.

Extreme caution must be observed in the interpretation of any results obtained in endemic or epidemic areas because of the possibility of inapparent infections occurring in vaccinated individuals.

Conversely, since vaccination is ordinarily performed immediately before the expected encephalitis season care must be taken in interpreting rises in neutralizing antibody titer in individuals with a clinical illness. In some instances the rise in titer as a result of vaccination may fortuitously coincide with the onset of clinical symptoms.

8. Laboratory Methods - The usual practice in this laboratory is to set up 60 complement-fixation and 40 neutralization tests five days per week. During 1948 approximately 230,000 albino "German" mice (a locally procured strain of unknown origin) have been used.

In the various papers to follow constant reference will be made to certain laboratory techniques. Consequently, to prevent repetition, the general techniques in use in this laboratory are here outlined in detail. In any instance where there has been deviation from these techniques it will be so indicated in the specific communication.

Blood specimens to be tested are drawn and processed aseptically, serum is separated as early as practicable and maintained until tested in deep freeze refrigerators supplemented with dry ice. No serum is tested until it has been in the deep freeze for at least 18 hours. Where serial survey specimens are examined, pre- and post-season sera are tested simultaneously. With a few exceptions, pre- and post-vaccination specimens are similarly tested at the same time. In some of the earlier work this was not possible and has been so indicated in the specific report.

a. Neutralization Tests - Intracerebral virus neutralization tests are carried out essentially according to the method recommended by the Neurotropic Virus Commission (65) employing mouse brain passage Nakayama strain. In early work infected mouse brain virus suspended in 10 percent normal rabbit-serum saline, centrifuged, quick-frozen, and maintained under dry ice refrigeration for periods of one to seven days was used. Because better virus potency and more uniformly satisfactory results obtained, fresh infected mouse brain material is now prepared and diluted in 20 percent sterile skim milk. Increasing dilutions of virus in 20 percent skim milk are added to equal volumes of the undiluted, non-inactivated serum to be tested. Virus-serum mixtures are incubated in a waterbath at 37° C. for 2 hours, chilled in an ice bath and kept cold until intracerebral inoculations can be carried out. Highest dilutions of virus are invariably inoculated first in each serum-virus series. Control LD_{50} titer of the virus preparation used in each test is determined by mixing identical virus dilutions with normal non-inactivated human serum from pre-tested, non-vaccinated donors newly arrived in the theater, and handling in the same manner as unknown sera. One control LD_{50} titration is included for each 10 to 14 sera tested, and inoculations made at the beginning, middle and end of each run. Maximum LD_{50} variation in these control titrations has been with rare exceptions less than 0.5 of one log and results are averaged in calculating LD_{50} titer. Known immune sera (rabbit, guinea-pig or human) are included as control sera. Four mice were inoculated with each dilution in early work and six mice per dilution is now standard and have been used in the majority of the tests to be reported. Deaths occurring in mice prior to onset of symptoms in control mice inoculated with equivalent virus dilutions are regarded as non-specific. The period of observation is 14 days. The degree of virus neutralization is determined by subtracting the reciprocal of the log LD_{50} of the test serum-virus from that of the control virus. The antilog of the obtained value gives the neutralization index. Usually indexes of 50 and greater are considered positive; 10 to 49, equivocal; and less than 10, negative.

b. Complement-Fixation Tests - Complement-fixation tests are performed in a manner essentially similar to that of Casals and Palacios (66). All sera are inactivated at 60° C. for 20 minutes immediately prior to testing. Unless otherwise stated, the lowest serum dilution tested is 1:4. Japanese B encephalitis antigen is prepared essentially according to Espana and Hammon (67) from infected mouse brain harvested just prior to death. Brain tissue is ground to a 20 percent concentration by weight in distilled water, shell-frozen in a mixture of dry ice and alcohol, and lyophilized. The dry powder is extracted by shaking in benzene in a mechanical shaker for short intervals over a 60 minute period, filtering under vacuum through a sintered glass filter, and repeating the extraction in the filter with several changes of benzene. After removal of traces of the solvent, the powder is transferred to vaccine bottles and stoppered with rubber vaccine caps. The partially processed antigen is stored in this form under dry ice refrigeration, or the preparation completed immediately. The final product is made by restoring to the original volume with normal saline, shaking, storing the suspension overnight at 5° C. and centrifuging at 13,000 r.p.m. in an angle head centrifuge refrigerated with dry ice. The supernatant fluid constitutes the stable fluid antigen and almost invariably contains one unit of antigen at a dilution of 1:64 (occasionally 1/128) in box titrations. Normal mouse brain for control is processed in an identical manner. Antigens prepared by this method have not shown anticomplementary effects even in undiluted form. Four units of virus antigen contained in 0.25 ml. are employed in the test. Three units of hemolysin and two exact units of complement titered in the presence of both virus antigen and normal mouse brain are used. Primary incubation is 16 to 18 hours at 5-8° C. Secondary complement titrations are carried out in the presence of antigens and normal saline solution. Tests are regarded as unsatisfactory if less than 1.7 or more than 2.5 units of complement are present as determined by the secondary titrations. After primary incubation, sheep cells sensitized at the time of

preliminary titrations and stored in the cold overnight are added, and the mixture incubated at 37° C. for 30 minutes. Serum controls are tested in 1:4 and 1:8 dilutions. Immune guinea pig serum prepared from infected hamster brain is employed routinely for positive control serum.

c. Virus Isolation - Attempts are made to obtain conclusive proof of virus etiology by isolation of the causative agent in fatal cases of encephalitis where brain tissue is available. Representative sections of control nervous tissue are suspended in 10 percent rabbit serum saline solution containing 500 to 1000 units of penicillin and 1500 units of streptomycin. After both low speed and high speed centrifugation, the suspensions are inoculated into young white swiss mice of American stock (3-5 day old mice are sometimes used) by the intracerebral and intraperitoneal routes. If the mice fail to show characteristic symptoms of disease in the first passage generation, two consecutive blind passages are carried out before reporting results as negative. When isolations are made, identification of the virus is done by appropriate serological tests with known hyperimmune sera.

9. Acknowledgment - The scope of the program reported upon is such that numerous agencies have been interested in various aspects. The program was initiated by the Commission on Virus and Rickettsial diseases, of which the most active members have been Dr. Albert Sabin, Dr. William Mc.D. Hammon, and Dr. John R. Paul. Most of the vaccine reported upon was prepared by the Army Medical Department Research and Graduate School and all of the assays on the vaccine have been conducted by this agency. Cooperative projects with Japanese physicians were made possible through the good offices of the Public Health and Welfare Section of the Supreme Command for the Allied Powers and frequently involved various prefectural Military Government teams. Japanese agencies cooperating in the project included the Japanese National Institute of Health, the various prefectural governments and local physicians. Several of the projects are reported jointly with other agencies. In the study of military groups full cooperation has been extended by Army, Navy and Air Force Surgeons and Commanders of various echelons in Japan, Korea, Okinawa and Guam.

II. Antibody Response in Japanese Children Following Japanese B Encephalitis Vaccination with Lyophilized Chick Embryo Type Vaccine

1. Materials and Methods - To ascertain the antibody response following administration of lyophilized chick embryo Japanese B encephalitis vaccine by subcutaneous and intradermal routes a project was undertaken in Japanese children at the Tokyo-to Shakujii-gakuen, a subsidiary orphanage of the Tokyo Orphanage, located in the suburbs of Tokyo, Japan. Ages of the children were between 2 and 7 years (mean 5.6 ± 1.42). They were divided at random into two groups, A and B, consisting of 22 and 24 children respectively.

Members of Group A received subcutaneously three doses of 1.0 ml each of the vaccine on the 24th and 29th of April and again on the 4th of May 1947. Children in Group B were injected intradermally with three doses of 0.1 ml. each on the same date.

The vaccine was lot No. 101-A, prepared from the Nakayama strain of Japanese B encephalitis virus by the Army Medical Department Research and Graduate School (57). Upon assay in mice (63) by AMDR&GS the 50% immunogenic dose (ID_{50}) was 0.006 ml.

Blood samples for neutralization tests were collected immediately prior to and ten days subsequent to vaccination (24 April and 14 May). Neutralization tests were conducted as outlined in the preceding paper as standard for this laboratory. It was not possible to conduct simultaneous tests.

2. Results - Sera of 4 of the 22 children in Group A and 3 of the 24 in Group B contained neutralizing antibodies against Japanese B virus in the pre-vaccination specimens and were, therefore, eliminated from the study. This percentage of initially positive neutralization tests was about that expected on the basis of previous studies (27) in this area.

For purposes of vaccine evaluation during a time when natural concommitant infection is extremely unlikely the development of one log or more in the neutralizing antibody content of a serum previously found to be devoid of neutralizing antibodies has been arbitrarily taken as a "satisfactory" response. The magnitude of the rise is recorded in all cases to permit regrouping of the data in any way desired to permit comparison with other studies.

As portrayed in Table 1, the effect of administration of chick embryo vaccine is exceptionally satisfactory in the subcutaneous group. Of the 18 individuals originally not demonstrating neutralizing antibodies and who subsequently received three doses of 1.0 ml. each of the vaccine subcutaneously,

a "satisfactory" response, a rise of one log or more, was demonstrated in fifteen (83%). "Satisfactory" amounts of neutralizing antibodies appeared in the sera of 8 of the 21 children (38%) who received three doses of 0.1 cc. each intradermally.

Table 1. Response in Serologically Negative Japanese Children Following Administration of Japanese B Encephalitis Vaccine

Route of Inoculation Group	Logs Protection[x] 3.0+	2.0+	1.7+[xx]	1.0+	Negative	Total Tested
A. Subcutaneous	5(28%)	13(72%)	14(78%)	15(83%)	3(17%)	18
B. Intradermal	5(24%)	8(38%)	8(38%)	8(38%)	13(62%)	21

[x] Totals are cumulative

[xx] Corresponds to Neutralization Index of 50 or more

3. Discussion - The response of the subcutaneously vaccinated children was far better than that of the children in the intradermal group. Satisfactory reactors to the vaccination were found in 83% in Group A, while only 38% of the children in Group B showed a positive rise. In the X^2 test P was 0.013.

Although there had been practically no known cases of Japanese B encephalitis in Tokyo during the lifetime of these children the presence of neutralizing antibodies in the sera of seven of the original group of 46 necessitates consideration of a possible anamnestic mechanism. The children considered as "negative" for the purpose of this study may possibly have been exposed to virus not sufficient in amount to elicit demonstrable antibodies, yet adequate to give an accentuated response on further stimulation. (Additional consideration of these groups is included in Section V of this report).

III. Field Trial of Japanese B Encephalitis Vaccine in Okayama Prefecture

To ascertain the effectiveness of Japanese B encephalitis vaccine in preventing morbidity or in modifying the course of the specific disease in an endemic or epidemic area it became necessary to set up a large scale field trial in an area where cases of the disease could be expected to occur and where suitable control groups could be studied.

1. Material and Methods - Location of trial - There is sufficient information contained in English summaries concerning Japanese B encephalitis to underline the fact that unpredictability of time of occurrence of significant numbers of cases is a prime characteristic of this disease. Although epidemics were probably experienced prior to that time, the 1924 epidemic remains as one of the three largest. 654 cases occurred in Okayama Prefecture and since that time this area (a part of the Setouchi District) on the Inland Sea of Japan has been considered as an endemic focus. For this reason Sabin chose to utilize Okayama as the locality for the vaccine trial beginning in 1946. However, relatively few clinical cases have occurred in this area since 1924. This is indicated in the following tabulation compiled from Kinoshita (69).

These are reported figures, based primarily on clinical diagnosis, but are sufficient to give a trend as to what might be expected. Few cases are recorded during the years 1940-1946.

Table 2. Reported Encephalitis Cases in Okayama-ken

	0-5[x]	6-10	11-20	21-30	31-40	41-50	51-60	61/	Total
1927	6	15	25	15	20	21	40	122	262
1928	0	2	1	2	1	3	3	9	21
1929	21	37	24	13	23	32	55	169	372
1930	1	6	10	6	8	10	12	42	95
1931	1	0	0	2	0	3	0	4	10
1932	7	6	7	4	8	2	14	41	89
1933	7	9	15	19	16	14	36	75	186
1934	0	1	2	3	0	9	7	6	19
1935	18	32	23	13	8	6	35	94	229
1936	4	3	5	4	1	3	5	13	38
1937	13	31	16	7	14	18	31	96	226
1938	13	12	15	5	6	6	12	42	111
1940	5	2	2	0	0	1	2	2	14
1941	3	2	1	3	0	0	3	5	17
Total	99	158	104	96	100	119	253	740	1689

x Japanese ages

Age Groups - Because of the frequency of cases and because surveys had indicated a high proportion of neutralizing antibodies in individuals over 10-14 years of age as a result of natural (clinical or inapparent) infections, it was decided to administer the vaccine primarily to the young age group. Some vaccine was given to individuals over 60 years of age.

Vaccine Administration - a. 1946 - During the period 10 June to 16 July 1946, Japanese physicians in collaboration with a group headed by Dr. Albert Sabin administered a full series (three doses of 1 ml each over a period of one month) of commercial mouse-brain type vaccine to 14,523 children in the prefecture of Okayama, Honshu, Japan. The children were between three and five years of age (American reckoning). No significant vaccine "reactions" were encountered. Assay values of this vaccine are not available but it consisted of many different lots. Post-season assay of two samples showed LD_{50}s of 0.013 and 0.077 (61) in contrast to the 1946 minimal requirement of 0.01 as a suitable ID_{50}. Pre-season assay values had all approximated this latter figure.

b. 1947 - Between 1 and 15 June 1947, Japanese physicians administered single recall doses to 13,257 of this same group of children. The American field representative was Dr. C. M. Wheeler. For various reasons, the dose used was 0.1 ml administered intracutaneously. This vaccine was of the chick embryo type, prepared by AMDR&GS, with pre-season assay values approximating an ID_{50} of 0.010.

c. 1948 - Between 15 and 30 June 1948 approximately 12,079 of this identical group received 1 ml subcutaneously of AMDR&GS chick embryo type vaccine, administered by prefectural health authorities. Between 7 June 1948 and 15 July 1948 an additional group of 14,207 received an initial immunizing series of three one cc doses of chick embryo AMDR&GS vaccine giving in 1948 a total immunized group of children of 25,247. The American field representative was Captain James P. Satterwhite, MC. The locations of the vaccinated groups are shown in Figure 2. Table III summates the three year vaccination program and gives the entire group figures for 1948.

Table 3. Vaccine Trial - Okayama-ken

	Type Vaccine	1st	2nd	3rd	Recall	Total No. Comp. Vacc. Children	Adults (over 60)	No. Localities Urban	Rural
1946 (Initial)	Mouse Brain 3 doses 1 cc over 1 month period	21,345	18,821	17,318	--	15,781 (14,523)[x]	1,537 (1,452)[x]	4	54
1947	Chick Embryo 0.1 cc ID				14,473	13,257	1,216	4	54
1948	Chick Embryo 1 cc SC				11,945	11,040	905	4	54
		x	x	x	x		x		
1948 (Initial)	Chick Embryo 3 cc SC over 1 mo. period	14,858	14,216	14,207		14,207		2	14
	1948 Total Vaccinated					25,247 26,152	905	4	65

[x]Parenthetical figures represent those records available in 1947. Other records had been lost or not prepared originally.

Vaccine was received as it became available from the ZI and transported by rail to Okayama in the original shipping containers refrigerated with wet ice. There it was either kept on wet ice, or kept in a deep freeze cabinet at -10° C. until used. A total of 63,250 ml. of vaccine was received. All vaccine to be used each day was rehydrated under supervision before 0730 hours, and kept in cans containing wet ice and sawdust until used, the remaining being discarded at the end of the day. The vaccine was administered by Japanese physicians, using 2, 5, and 10 ml. syringes, with a freshly sterilized needle for each child. The child's arm was cleansed with 70% alcohol and 1.0 ml. vaccine was given subcutaneously in the triceps area. The reactions recorded from the first dose were: slight fever, immediate local reddening, occasional headache, nausea, vomiting and dizziness. None of these reactions was noted during the remainder of the program. The vaccine administered in 1948 was of several lots and each dose given any individual child was of a different lot number. Samples (total of 1,050 ml.) of most of the lots used were returned to AMDR&GS for reassay. Pre- and post-season assays are shown in Table 4 (73).

Records - A card file in Japanese and English is maintained for each vaccinated individual showing age, location by school, locality and home address. Type and lot number of vaccine is recorded per locality. Detailed maps and census data have been made available by prefectural authorities.

Case evaluation - Similar information is recorded for each suspect case of Japanese B encephalitis in Okayama prefecture. During 1946 each suspect case was carefully evaluated by American and Japanese workers. During 1947 Japanese health authorities maintained a similar program but serological examination was carried out in a relatively few instances. During 1948 American and Japanese field workers saw and obtained blood samples on approximately 90% of all suspect cases except those in whom death occurred early in the illness. Particular attention was directed to any cases in the age group under study. Serial complement-fixation and neutralization tests were carried out in Tokyo. The sera were maintained under dry ice refrigeration until such time as simultaneous testing was possible. A rise in one or both tests coupled with clinical evidence of a disease of the central nervous system was considered diagnostic. Details of the tests have been described in an earlier paper.

Occurrence of cases - a. 1946 - Only one case of Japanese B encephalitis was reported to have occurred in the entire prefecture, in a 41 year old woman.

Table 4. Results of Reassay of Japanese Encephalitis Vaccine Shipped Back from Pacific Theater to AMDR&GS - August 1948

Vaccine Lot	Assay before Shipment[x]		Expiration Date	Assay after Return	
	Date	MID (cc.)		Date	MID (cc.)
205 A	1/6/48	0.008	10/1/48	8/31/48	0.011
208 A	2/12/48	0.017	10/1/48	8/31/48	0.050
208 B	2/12/48	0.004	10/1/48	9/11/48	0.035
212 A	2/24/48	0.012	10/1/48	8/31/48	0.009
212 D	2/12/48	0.012	10/1/48	9/11/48	0.032
214-17 A	3/30/48	0.011	10/1/48	8/31/48	0.025
214-18 A	5/14/48	0.021	10/1/48	8/31/48	0.031
217-23 A	6/11/48	0.010			
217-23 B	4/30/48	0.17	10/1/48	8/31/48	0.046
223-34 A	5/14/48	0.020	10/1/48	8/31/48	0.042
223-35 A	5/14/48	0.019	10/1/48	8/31/48	0.019
224 A	6/11/48	0.019			

[x] The acceptable ID_{50} in 1948 was 0.02 ml. (63).

Table 5. Case Occurrence Japanese B Encephalitis in Okayama-Ken in 1947 Relationship to Population and Vaccination

Place	Census and ages 1948 5	6	7	Total	Age of 1947 Cases 4	5	6	Total Vaccinated in both 1946 and 1947	Total Non-Vaccinated
Okayama-shi	3,218	3,247	3,153	9,618		2	1	614	
Kurashiki-shi	1,169	967	761	2,897	1		1	1,144	
Kanura-cho (Oda-gun)	236	221	240	697			1	229	
Total	4,623	4,435	4,144	13,202		6		1,987	11,694
Correction factors to approximate total population figures for 1947				188 (all non-vacc.) 291					
Estimated 1947 total				13,681					

Expected cases in vaccinated at rate of total = 1,987/13,681 x 6 = 0.87

Expected cases in vaccinated at rate of unvaccinated = 1,987/11,694 x 6 = 1.01

Actual cases in vaccinated group = 0


JAPANESE B ENCEPHALITIS
IN OKAYAMA-KEN, 1946-1948
LEGEND
LOCATION AND TOTAL NUMBER OF JAPANESE B ENCEPHALITIS VACCINATIONS. FIRST COURSE OF IMMUNIZATION 1946; BOOSTER DOSES 1947 AND 1948.
LOCATION OF SCHOOL IN JAPANESE B ENCEPHALITIS VACCINATION PROGRAM AND TOTAL VACCINATED. FIRST COURSE OF IMMUNIZATION 1948.
PREFECTURAL BOUNDARIES
GUN AND SHI BOUNDARIES
TOMATA GUN
MANIWA GUN
ATETSU GUN
KUME GUN
TSUYAMA SHI
KATSUTA GUN
HIDETA GUN
JOBO GUN
MITSU GUN
AKAIWA GUN
KAWAKAMI GUN
WAKE GUN
IMBECHO
SHIRAISHI
SOJACHO
KIBI GUN
JODO GUN
OKU GUN
OKA-YAMA SHI
TSUKUBO GUN
SHITSUKI GUN
KURASHIKI SHI
FUNAHOCHO
ODA GUN
TAMASHIMACHO
KOJIMA GUN
ASAKUCHI GUN
SATOSHOSON
KASAOKACHO
TAMANO SHI
SHODO-SHIMA
KOJIMA SHI
INLAND SEA
TAKAMATSU SHI
SCALE
MILES
0
5
10

FIG. 2

Table 6. Case Occurrence Japanese B Encephalitis in Okayama-ken in 1948

Relationship to Population

Place	5	6	7	8	9	Total	Cases	Init. Vacc. 1948	Recall 1948	Total Vacc. 1948	Total Cases in Vacc.	Total Non-vacc. 1948	Total Cases in Non-Vacc. Group
a. Serologically confirmed cases-													
Okayama-shi	(36R)★ 3,218	(38R) 3,247	3,153	(66R) 2,716	2,201	14,535	3	5,948	558	6,506	1	8,029	2
Kojima-shi	847	790	803	740	(22R) 541	3,621	1		476	476		3,145	1
Soja-cho (Kibi-gun	220	217	247	191	(19R) 182	(63R) 1,057	2		343	343		714	2
Kasaoka-cho (Oda-gun)	488	441	467	387	(59R) 272	2,055	1		515	515		1,540	1
Tamashima-cho (Asakuchi-gun)	806	(94R) 590	521	563	(62R) 629	3,109	2		724	724		2,385	2
Imbe-cho (Wake-gun)	170	158	126	(100R) 122	112	688	1		106	106		582	1
Funaho-cho (Asakuchi-gun)	135	179	(69R) 166	115	99	744	1	466		466		278	1
Totals						25,809	11			9,136	1	16,673	10

Expected cases in vaccinated group at rate of total = 9,136/25,809 x 11 = 4.0

Expected cases in vaccinated group at rate of unvaccinated = 9,136/16,673 x 10= 5.5

Actual cases in vaccinated group = 1

b. Cases with typical history and clinical picture - no blood samples available -

Kurashiki-shi	(50F) 1,169)	967	761	774	927	4,598	1	2,145	1,014	3,159	1,439	1
Satosho-shi (Asakuchi-gun)	(47F) 196	171	154	167	143	831	1		165	165	666	1
Shirashi-son (Mitsu-gun)	(25R)					150	1	31		31	119	1
Tsuyama-shi	1,256	1,279	1,194	(86F) 1,199	1,285	6,213	1		717	717	5,496	1
Totals						11,792	4			4,072	7,720	4

Expected cases in vaccinated group at rate of total = 4,072/11,792 x 4 = 1.38

Expected cases in vaccinated group at rate of unvaccinated = 4,072/7,720 x 4 = 2.1

Actual cases in vaccinated group = 0

. Summation of all cases in 5-9 age group in localities where vaccination was accomplished -

Grand Totals	37,601	15	13,208	24,393	14

Expected cases in vaccinated group at rate of total = 13,208/37,601 x 15 = 5.27

Expected cases in vaccinated group at rate of unvaccinated = 13,208/24,393 x 14 = 7.6

Actual cases in vaccinated group = 1

★ Parenthetical code over population figure indicates one case in that age group. The number given refers to the master list. F indicates a fatal outcome, R indicates recovery.

b. 1947 - 72 cases diagnosed on a clinical basis were reported. Of these, four occurred in April, May and June. On the basis of present knowledge concerning the rather sharp seasonal distribution of cases and the fact that such early cases have seldom if ever received laboratory confirmation, these have arbitrarily been eliminated from consideration. While some cases may have been missed, other cases were undoubtedly incorrectly diagnosed. In at least one district, 4 of 5 cases reported in September were in individuals from 61 to 75 years of age; none was serologically proved. Positive serologic results were obtained in four of 18 cases tested during 1947. Of the 68 possible cases remaining 24 occurred among children 9 years of age or younger. The incidence was extremely spotty, and in only three towns were there more than two cases per town reported. There were no suspect cases in the vaccinated group. In three localities where there were vaccinated individuals, 6 cases were reported in children of an age range (4-6 years) comparable to the immunized group. In Table 5 additional data is presented. In arriving at the numbers at risk 1948 population data was corrected to approximate 1947 figures.

c. 1948 - There were 100 cases of suspect Japanese B encephalitis reported in Okayama-ken. In 37 instances, complement-fixation titers showed a definite rise. 27 additional cases were considered clinically authentic, giving a total of 64 patients in all age groups throughout the entire area but with a definite concentration in the south. 23 cases were observed in the 5 - 10 age group (18 with serological evidence of disease) and 15 bonafide cases are considered to have occurred in the 5 - 9 age group in localities where there were vaccinated individuals of the same ages (See Fig. 2). A summary is given in Table 6. The table is divided so that calculations can be made on the basis of serological proof and on clinical impression. It will be noted that 3 of the 4 cases in the last named group were fatal while none of the serologically proved cases was fatal. This gives an overall mortality of 3/15 (20%) which conforms to the expected rate in this age group.

A single case occurred in the vaccinated group. An eight year old boy (66R) was given an entire course of vaccine in 1948, completing the course seven weeks prior to onset of symptoms (30 August 1948). In comparison to other cases occurring in the area his clinical course was considered moderately severe. There was no apparent modification of the acute phase. Clinical recovery began on the eighth day and was rapid and complete. Simultaneous complement-fixation tests showed a 1/ at 1:8 on the initial sample and 4/ at 1:16 on the second sample. Six separate lots of vaccine were used for vaccination of the group in which this case occurrence so that he received vaccine from 3 separate lots.

2. Discussion - In attempting to draw conclusions as to vaccine efficacy among immunized individuals in Okayama-ken in the face of the relatively small number of cases which have occurred over the three year period, careful study must be made of all localities containing vaccinated groups and also containing cases of Japanese B encephalitis occurring in an identical age group (4-6 in 1947; 5-9 in 1948). The inclusion of the four and five year group makes individual school studies impossible to attain but is necessary both because of occurrence of cases and because large numbers of the group originally vaccinated in 1946 fall into this age category. The small number of people over 60 years of age originally vaccinated in 1946 has now fallen to 905 and is too small to warrant statistical treatment. No cases of Japanese B encephalitis are known to have occurred in this elderly vaccinated group.

On the basis of the figures shown in Table 5, it is calculated that 0.87 cases would have been expected in 1947 in the vaccinated group at the rate of the total (6 cases in 13,681); and that at the rate of case occurrence in the unvaccinated group (6 cases in 11,694), 1.01 cases would have been expected among vaccinated individuals. The fact that none occurred is obviously of little significance.

If the 15 cases occurring among the 5-9 year old children in 1948 are considered as occurring in an epidemiological unit of 37,601 individuals of which 13,208 were vaccinated, an incidence of 5.27 cases in the vaccinated group would be anticipated if the two groups were identical. (13,208/37,601 x 15 = 5.27). Actually one case was seen. A less acceptable method of measurement, the number of cases in the vaccinated group at rates of non-vaccinated, is 7.6 cases expected. (13,208/24,393 x 14).

In five instances cases occurred in schools containing vaccinated groups. The census of the first four grades of these four schools was 4,436, of which 1926 individuals has been vaccinated in 1948, either initially or recall. (This small group of cases contains, of course, the single case reported in a vaccinated individual). On the same basis as above 2.1 cases would have been expected in the vaccinated group at the rate of total incidence (1,926/4,436 x 5 = 2.1), while at the rate of cases in the unvaccinated 3.0 cases would have been anticipated (1,926/2,511 x 4 = 3.0).

3. Summary - Over a three year period Japanese B encephalitis vaccine has been administered to children in Okayama prefecture. Both the dosage schedule and type of vaccine have varied. During the first year (1946) only one case of encephalitis occurred in the entire prefecture in an individual outside the age group under study. During the second year a total of 6 cases occurred in the prefecture in the age group under study with none in the vaccinated group. During the third year (1948) 64 cases were observed in the entire area. One non-fatal case occurred in the vaccinated group while case expectancy on the basis of the overall occurrence in the age group under study was 5.27. On the basis of case occurrence in the non-vaccinated the anticipated number of cases in the vaccinated group was 7.6. The results are merely suggestive that the vaccine is of some value.

IV. Serologic Response to Repeated Immunization With Japanese B Encephalitis Vaccine

In the previous communication it was pointed out that while a vaccine field trial carried out in Okayama-Ken over a three year period suggested that numbers of cases of Japanese B encephalitis in immunized individuals might be reduced as compared with those in non-vaccinated individuals, case incidence in comparable age groups was too small to admit drawing of definite conclusions. A supporting method of testing vaccine efficacy lies in the measurement of antibody response following administration of the vaccine providing particular care is taken to eliminate or to weigh the degree to which the extraneous factor of actual infection (sub-clinical or inapparent) may contribute to the serologic picture in the vaccinated individuals. Distinct differences in specific antibody content should be demonstrable between homogenous groups of individuals in identical epidemiological units where the only factor not held in common is the administration or non-administration of vaccine.

Practically no information is available concerning the effect of repeated recall doses administered over a period of time to a large group of immunized individuals. This report covers results of serologic examination in children from Okayama-ken (as outlined in Section III preceding) who have received repeated inoculations over a period of three years with vaccines of varying type, potency and route of administration.

1. Controls - In order to establish the influence of naturally occurring experience with the living virus, consideration must first be given to the serological pattern obtaining in unvaccinated individuals of comparable ages and geographical situations. Sabin et al (61) in 1946 found that none of 10 children from Tsuyama-shi nor of 10 children of the same 3-5 year age group from the rural areas of Kume-son and Miho-cho showed demonstrable neutralizing antibodies prior to vaccination. In November of the same year, Deuel and Bawell (27) examined serum specimens from 70 children ranging in age from less than one to 9 years. Samples were obtained in Okayama-shi and in the rural area of Seto-cho. None of 29 individuals less than 5 years of age had neutralizing antibodies against the Nakayama strain of virus, while only 5 of 41 sera (12 per cent) from non-vaccinated children of the 5-9 year age group were positive. These figures are in marked contrast to those obtained during the same period in specimens from Okinawa and other areas where the disease had been present in epidemic form in the preceding summer.

Pre-season blood specimens were again obtained in early March 1948 from unvaccinated children in urban and rural areas where vaccinations had been carried out in 1946 and 1947. Individuals tested were between the ages of 5 and 9, inclusive; in the Kurashiki-shi and Saidaiji-cho areas the 8 and 9 year old children predominated. Results showed that from 75 to 88 per cent still lacked neutralizing antibodies (Table 7). Neutralization tests were carried out by the method described earlier.(Additional consideration of these data will be found in Section V.)

Table 7. Results of JBE Virus Neutralization Tests with Sera from Non-Vaccinated Japanese Children (5-9 years) in Okayama-Ken, March 1948

	Positive	Equivocal	Negative
Okayama-shi	3/24 - 13%	3/24 - 13%	18/24 - 75%
Kurashiki-shi	3/25 - 12%	0/25 - 0%	22/25 - 88%
Saidaiji-cho	2/20 - 10%	3/20 - 15%	15/20 - 75%
Seto-cho	2/23 - 9%	1/23 - 4%	20/23 - 87%

Essentially similar results were obtained on the same sera tested independently in the Japanese National Institute of Health (70) using the Kalanina strain of virus.

These surveys with non-vaccinated children demonstrate clearly that on the basis of neutralization tests, individuals comparable in age to those in vaccinated groups and living in the localities under study show little evidence of experience with the virus of Japanese B encephalitis. This is not surprising in view of the absence of clinical cases in this age group during 1946 and the relatively few cases in 1947. Any significant difference in the serologic pattern among vaccinated individuals, therefore, can probably be ascribed to influence of vaccine administration rather than solely to naturally occurring infections.

2. Method - To determine the influence of mass immunization with Japanese B encephalitis vaccine upon antibody response, more than 200 blood specimens were drawn from children (5-8 years) in Okayama-shi and Kurashiki-shi. These individuals had been vaccinated with a full course of mouse brain preparations in 1946 and had received a 0.1 cc. intracutaneous recall dose of chick embryo vaccine in 1947. Specimens were obtained on the same date (early March) that samples were taken from the non-vaccinated children mentioned earlier. After bleeding, approximately one-half of the group received a 0.1 cc recall dose given intracutaneously while the remainder received a 1.0 cc. subcutaneous dose. Vaccine employed in the trial was of a single lot, 205A, AMDR&GS chick embryo type. The initial assay was ID_{50} 0.008 and on reassay 5 months later was 0.011 (68). Post-vaccination specimens were obtained 20 days later. Sera were separated and stored in the frozen state until tested. Tests methods have been described previously.

Table 8. Complement Fixation and Neutralizing Antibody Response Following Administration of Intracutaneous or Subcutaneous Recall Doses in Previously Immunized Children

Route and Dose	Specimen	Complement Fixation Positive	4/	3/	2/	1.7/	1.0/	Neg.	Total Tested
Intradermal 0.1 ml.	Pre	-	1(1%)	16(18%)	31(35%)	35(40%)	51(58%)	37(42%)	88
	Post	30/102(29%)	35(35%)	77(77%)	89(89%)	91(91%)	94(93%)	7(7%)	101
S.C. Subcutaneous 1.0 ml. 1.0	Pre	-	6(6%)	13(12%)	36(34%)	44(42%)	80(75%)	26(25%)	106
	Post	52/97(54%)	38(40%)	84(89%)	90(96%)	90(96%)	92(98%)	2(2%)	94
Combined Totals	Pre	-	7(3%)	29(15%)	67(35%)	79(40%)	131(68%)	63(32%)	194
	Post	82/199(41%)	73(37%)	161(82%)	179(92%)	181(93%)	186(95%)	9(5%)	195

3. Results - Brief inspection of the data presented in Table 8 makes it apparent at once that in early March 1948 there existed a distinct difference in the percentage of previously immunized individuals showing neutralizing antibodies as compared with non-vaccinated children in the same localities (Table 7). Any difference attributable to age (greater length of exposure to virus contact) would favor a preponderance of positives in the non-vaccinated group since more than three-fourths of these children from Okayama and Kurashiki were 8 years of age or older while in the vaccinated group more than three-fourths were less than 8 years old. As can be seen, 40 per cent (79/194) of all previously immunized children showed positive neutralization indexes with an additional 27 percent in the equivocal range, while only 12 per cent (6/49) of the control group from Okayama and Kurashiki fell in this category (6 per cent equivocal). This difference may be found to be highly significant.

Following administration of the recall dose, regardless of dosage or route employed, the difference is seen to be still further exaggerated between the proportion of individuals showing positive neutralization indexes in the vaccinated group as compared with those in the non-vaccinated group. The proportions of both equivocal and negative indexes are strongly reduced with a corresponding increase in the positive category. Ninety per cent and 96 per cent of the immunized group show positive neutralization indexes, respectively. While the route and dosage of inoculum used does not appear to exert a significant influence on the total proportion of positive neutralization indexes induced in response

to recall vaccination, a significantly greater percentage of individuals have been found to show positive complement fixation responses after subcutaneous inoculation with 1.0 cc. of vaccine (54 per cent) than after intracutaneous vaccination with 0.1 cc (29 per cent). Positive reactions with both groups ranged in titer from 1:4 to 1:16

4. Discussion - It has been demonstrated previously that a high order of neutralizing antibody response in Japanese children may be induced by an initial series of inoculations with either mouse brain or chick embryo JBE vaccines of adequate potency. While work by Warren (62) and by this laboratory indicate that either the intracutaneous or the subcutaneous method of administration of recall doses is almost equally effective in stimulating neutralizing antibody production, there is definite evidence to support the view that the subcutaneous method is preferable for the primary immunization of children with JBE vaccine of the chick embryo type. It has also been found (Section VII) that in adult military personnel, at least, neither route of administration of recall doses will induce a complement fixation response comparable to that seen in children of the present series.

Hammon (64) in 1947 also demonstrated a significant (about 50%) increase in neutralizing antibodies following recall vaccination in the Okayama group by the intracutaneous route. Results obtained in March 1948 show that a considerable number of the previously immunized children have no demonstrable neutralizing antibodies but that a prompt increase in the number of positive neutralization indexes occurs following administration of even a small recall dose of potent vaccine. However, a small group has been found to show no antibodies after repeated vaccination. Whether such individuals are more susceptible to the disease than the group showing ready antibody response to vaccination remains an unanswered question.

From the fact that only 41 per cent of the previously immunized individuals showed positive neutralization indexes in pre-season, pre-recall dose specimens, it can be assumed that either a large part of the vaccine administered in 1946 and 1947 was lacking in potency, or that circulating antibodies persist for relatively short periods of time following inoculation with dead virus preparations. The prompt antibody response to injection of recall doses of vaccine appears to indicate an anamnestic type of reaction and thus would support the second view.

5. Summary - A significantly higher proportion of positive neutralization indexes for Japanese B encephalitis virus was found in early March 1948 among Japanese children previously vaccinated in 1946 and 1947 with preparations of varying type, potency, dosage and route of administration than among individuals of comparable ages and geographic locations who had not received vaccine. Forty-one per cent of such previously immunized children exhibited titers in the positive range (neutralization index of 50 or greater) compared to 12 per cent in the non-vaccinated group.

Subcutaneous administration in 1948 of 1 cc. of potent chick embryo type vaccine to the previously vaccinated group increased the proportion of positive neutralization indexes to 96 per cent, with 54 per cent showing positive complement fixation titers of 1:4 or higher 20 days after inoculation. Intracutaneous administration of 0.1 cc. of the same vaccine induced an increase to 90 per cent in percentage of positive neutralization indexes; 29 per cent showed positive complement fixation reaction.

V. Notes on Possible Vectors, Inapparent Infections, and Persistance of Antibodies in Okayama-Ken, 1948

The first serologically confirmed case of Japanese B encephalitis in Okayama-ken during the 1948 season had an onset date of 9 August. The last serologically confirmed case of the season had an onset date of 22 September. In Okayama-shi 17 suspect cases were recorded and, of these, 12 are considered to be bonafide cases. The approximate location of these 12 cases is shown in Figure 3. The location of vaccinated groups is also indicated. Similar data is available for all other areas of Okayama-ken but is not presented for reasons that will become apparent.

1. Possible Vectors - During the period 25 July to 3 September of 1948, members of the 207th Malaria Survey Detachment collected and processed mosquitoes in the Okayama area for attempts at virus isolation. A total of 44,707 adult female mosquitoes were obtained. Approximately 75% were Culex tritaeniorhynchus with Anopheles hyrancus sinensis being the second most prevalent. These catches were transmitted to Dr. W. Mc.D. Hammon (The Hooper Foundation) for inoculation, since it was considered that any isolations made in Japan would be suspect because of the ever present possibility of activating a latent infection in the test animals.

On 2 November 1948 Hammon reported than on initial passage an isolate of Japanese B virus had been made from one lot of C. tritaeniorhynchus (40-C). This collection had been made on 9 August, which coincides with the onset date of the first confirmed case of Japanese B encephalitis in the entire prefecture

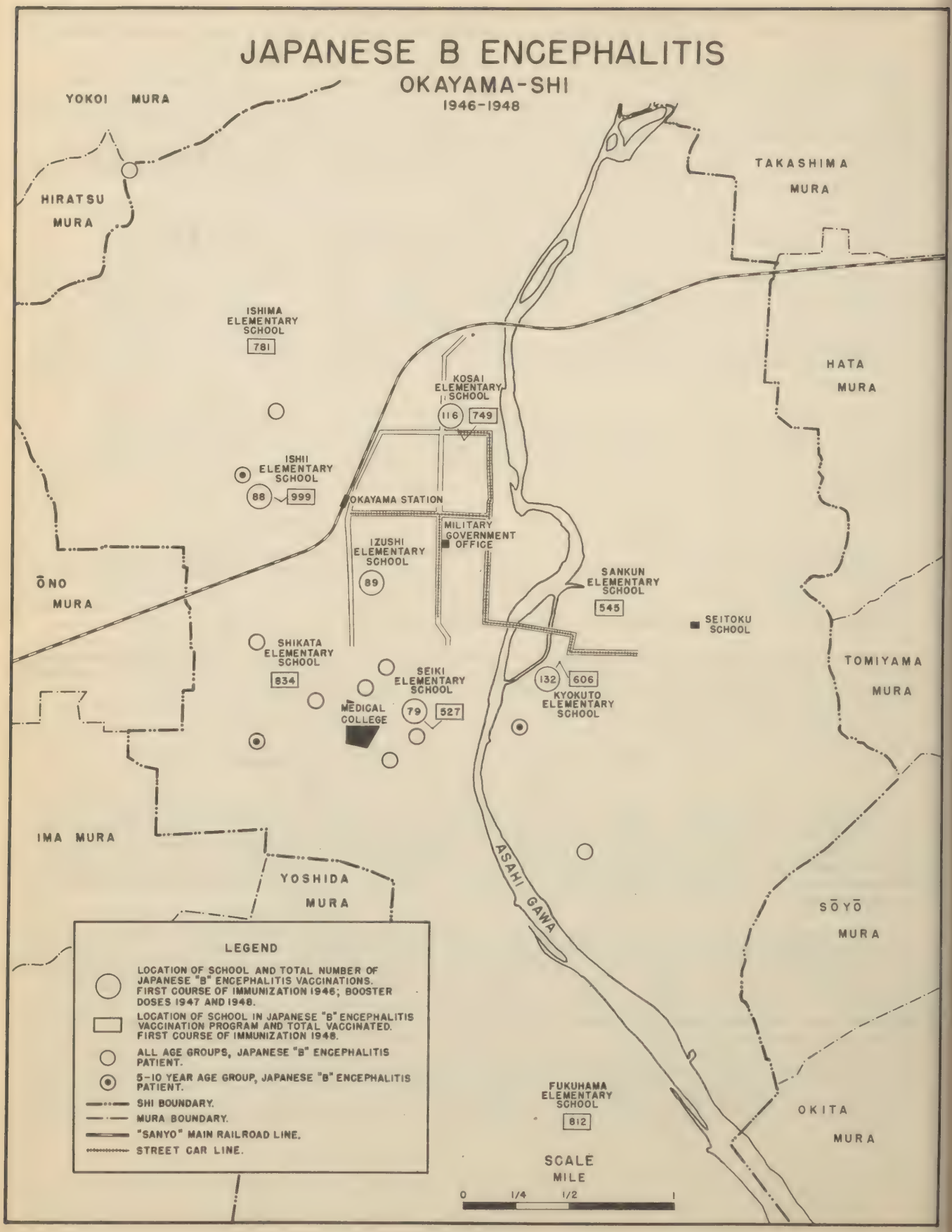

JAPANESE B ENCEPHALITIS
OKAYAMA-SHI
1946-1948
YOKOI MURA
HIRATSU MURA
TAKASHIMA MURA
HATA MURA
ISHIMA ELEMENTARY SCHOOL
781
KOSAI ELEMENTARY SCHOOL
116
749
ISHII ELEMENTARY SCHOOL
88
999
OKAYAMA STATION
MILITARY GOVERNMENT OFFICE
IZUSHI ELEMENTARY SCHOOL
89
SANKUN ELEMENTARY SCHOOL
545
SEITOKU SCHOOL
ŌNO MURA
SHIKATA ELEMENTARY SCHOOL
834
SEIKI ELEMENTARY SCHOOL
MEDICAL COLLEGE
79
527
132
606
KYOKUTO ELEMENTARY SCHOOL
TOMIYAMA MURA
IMA MURA
YOSHIDA MURA
ASAHI GAWA
SŌYŌ MURA
FUKUHAMA ELEMENTARY SCHOOL
812
OKITA MURA
LEGEND
LOCATION OF SCHOOL AND TOTAL NUMBER OF JAPANESE "B" ENCEPHALITIS VACCINATIONS. FIRST COURSE OF IMMUNIZATION 1946; BOOSTER DOSES 1947 AND 1948.
LOCATION OF SCHOOL IN JAPANESE "B" ENCEPHALITIS VACCINATION PROGRAM AND TOTAL VACCINATED. FIRST COURSE OF IMMUNIZATION 1948.
ALL AGE GROUPS, JAPANESE "B" ENCEPHALITIS PATIENT.
5-10 YEAR AGE GROUP, JAPANESE "B" ENCEPHALITIS PATIENT.
SHI BOUNDARY.
MURA BOUNDARY.
"SANYO" MAIN RAILROAD LINE.
STREET CAR LINE.
SCALE
MILE
0
1/4
1/2
1

FIG. 3

and precedes by 6 days the onset of the first confirmed case in Okayama-shi. This collection was made in a cow-barn in Okayama-shi, exact location not known other than that it was near the city limits.

2. Inapparent Infections - To estimate the magnitude of inapparent infections blood specimens were obtained from groups of unvaccinated children in Okayama, Kurashiki, Saidaiji and Seto in early July. A portion of the same group from Okayama City (Seitoku School) tested in March was included among those bled in July. Sera were stored under dry ice refrigeration. Post-season blood specimens from these same individuals were again obtained in late September, processed and maintained in the frozen state. Both complement fixation and virus neutralization tests were carried out on paired July and September specimens simultaneously. Results are summarized in Table 9. Table 10 lists changes found in duplicate specimens for each area.

Table 9. Results of Complement Fixation and Neutralization Tests for Japanese B Encephalitis Virus Antibodies with July and September Sera from Non-Vaccinated Japanese Children, 1948

Locality	Specimen	Complement Fixation Positive	4/	3/	2/	1.7/	1/	1.0	Total Tested
Seto-cho	July	0	0	2(5%)	31(67%)	3(6%)	10(16%)	33(77%)	43
	Sept.	0	0	3(6%)	4(8%)	4(8%)	8(9%)	39(83%)	47
Kurashiki-shi	July	0	2(4%)	4(8%)	8(18%)	9(20%)	12(27%)	33(73%)	45
	Sept.	0	1(2%)	6(12%)	10(22%)	10(22%)	17(37%)	29(63%)	46
Saidaiji-cho	July	1/49(2%)	0	7(15%)	9(19%)	9(19%)	15(32%)	32(68%)	47
	Sept.	2/38(5%)	2(6%)	11(30%)	11(30%)	12(33%)	13(36%)	23(64%)	36
Okayama-shi (Seitoku)	July	0	0	3(6%)	10(19%)	11(21%)	16(30%)	37(70%)	53
	Sept.	9/51(18%)	0	4(9%)	15(31%)	21(42%)	27(57%)	20(43%)	47

Most of the "positives" are low in titer when compared to the levels obtained in clinical cases. The highest neutralization index in this group was 4,000. Complement fixation titers as high as 1:16 were obtained. These levels of neutralizing antibodies and complement fixing antibodies are definitely lower than were demonstrated in individuals in the Tokyo area following the 1948 epidemic. The possible epidemiological significance of this apparent difference remains to be assessed.

From these data it can be seen that in so far as a change from negative to positive complement fixation reactions and neutralization indexes can be relied upon as a measurement of inapparent infection, significant exposure to the virus of Japanese B encephalitis virus occurred among the groups tested in Okayama City and, to a lesser extent, in Saidaiji-cho. Since none of these children had ever received JBE vaccine, there appears to be small doubt that contact with living virus under natural conditions was responsible for the changes in antibody content observed. However, the proportion of non-vaccinated children showing positive JBE antibody content in post-season specimens is still far short of that seen in comparable groups of previously immunized individuals subsequent to administration of recall doses of vaccine. This finding strengthens the view that vaccine administration alone was operative in the production of significant differences in antibody content between the non-vaccinated and previously vaccinated groups.

Persistance of Antibodies Following Vaccination - Blood specimens were obtained in November from vaccinated children in Sankun School located about one mile from Seitoku-School where post-season specimens (listed in Table 3) had been drawn 26 days earlier from non-vaccinated individuals. This group of children had not been vaccinated prior to 1948 and had received the full three 1.0 ml of vaccine in June. Five lots of chick embryo vaccine, varying in assay values from 0.004 to 0.021 (73), had been used. Of 49 sera drawn in mid-November, only 3 (6%) were positive to complement-fixation at a dilution of 1:4 or higher. Four additional sera were positive in 1:2 dilution only, making a total of 7/49 (14%) positive at this period in spite of vaccination and presumed exposure to JBE virus during the late summer and fall. Neutralization tests with these sera are not available at the time of this report.

Projection - These data are presented because they form a base line for other studies now initiated with the aim to clarify the persistence of antibodies. Since other children in Okayama-ken may have been exposed to amounts of virus insufficient in amount to elicit demonstrable antibodies, yet adequate to give an accentuated response on further stimulation, it will be necessary to evaluate carefully the effect of a single dose of vaccine on a previously unvaccinated group prior to the 1949 season. The data to be obtained thereby will permit a more logical evaluation of the effects of vaccination on the overall immunological response, since any measurable response to a single dose of vaccine in a previously unvaccinated individual is probably indicative of previous experience with the virus, regardless of the absence of serologic evidence prior to the test dose.

Table 10. Summary of Changes in Neutralization Indexes in Paired Serum Specimens (July and September, 1948) from Non-Vaccinated Japanese Children, Okayama-Ken

Serum Category	Seto-cho	Kurashiki-shi	Saidaiji-cho	Okayama-shi
Neg. July Neg. Sept.	25/30 - 83%	24/30 - 80%	21/23 - 91%	18/31 - 58%
Neg. July Equiv. Sept.	4/30 - 13%	6/30 - 20%	0/23 - 0%	4/31 - 13%
Neg. July Pos. Sept.	1/30 - 3%	0/30 - 0%	2/23 - 9%	9/31 - 29%
Neg. July No. Spec. Sept.	3	3	9	6
Equiv. July Neg. Sept.	5/7 - 71%	1/2 - 50%	2/4 - 50%	1/4 - 25%
Equiv. July Equiv. Sept.	0/7 - 0%	1/2 - 50%	1/4 - 25%	0/4 - 0%
Equiv. July Pos. Sept.	2/7 - 29%	0/2 - 0%	1/4 - 25%	3/4 - 75%
Equiv. July No. Spec. Sept.	0	1	2	1
Pos. July Neg. Sept.	[x]2/3 - 67%	0/9 - 0%	0/9 - 0%	0/9 - 0%
Pos. July Neg. Sept.	0/3 - 0%	0/9 - 0%	0/9 - 0%	[xx]2/9 - 22%
Pos. July Pos. Sept.	1/3 - 33%	9/9 --100%	9/9 -100%	7/9 - 78%
Pos. July No Spec. Sept.	0	0	0	2
No. Spec. July Neg. Sept.	7	4	0	1
No. Spec. July Equiv. Sept.	0	0	0	0
No. Spec. July Pos. Sept.	0	1	0	2

[x]

	Neut. Index July	Sept.
(1)	1000	8
(2)	320	5

[xx]

	Neut. Index July	Sept.
(1)	80	32
(2)	2000	32

VI. Antibody Response in Military Personnel Following Initial Administration Of Japanese B Encephalitis Vaccine (Lyophilized Chick Embryo Type)

The present study was undertaken to ascertain and compare the effectiveness of subcutaneous and intradermal vaccination of American Military personnel, using the chick embryo vaccine furnished by AMDR&GS in 1947, as manifested by the neutralizing antibody response elicited.

1. Material and Methods - Lyophilized chick embryo type vaccine (57), Lot No. 101-A, was used. The ID_{50} prior to shipment from AMDR&GS was 0.006 (63.68). All vaccine was administered within six hours subsequent to rehydration. Volunteers were obtained from American Military Personnel billeted in a single large building in Tokyo, housing some 4,000 troops. Individuals who had not previously received any type of Japanese B encephalitis vaccine were selected and divided into three equal groups.

Group A received a total of 3.0 ml. of vaccine given by the subcutaneous route in doses of 1.0 ml. on the first (15 April 1947), eighth, and twenty-ninth days. The ages of this group ranged from 18 to 24 with a mean of 18.9 $\pm$ 1.02.

Group B received a total of 0.3 ml. of vaccine given by the intradermal route in doses of 0.1 ml. on the first (16 April 1947), seventh, and twenty-ninth days. The ages of this group ranged from 18 to 27 with a mean of 18.6 $\pm$ 1.22.

Group C was used as a control group and received 0.1 ml. of typhoid vaccine intradermally at the initiation of the experiment. Since they are also being used as part of another project blood samples were obtained from this group two weeks after the initial typhoid administration only.

Blood samples were obtained prior to immunization and two weeks following the last dose. These were collected and handled in the manner described in the previous publication. Simultaneous testing of pre-and post-vaccination sera was not possible since time and availability of animals would not permit. Hence, the results presented constitute a number of different tests. On initial testing no member of the three groups showed neutralizing antibodies for the virus of Japanese B encephalitis.

After results of the first tests had been obtained on Group B additional vaccine was administered. This consisted of one dose of 0.1 ml. intradermally and two doses of 1.0 ml. each subcutaneously, inoculated on the 47th, 65th, and 78th days respectively after the first dose of the original administration. On the 100th day (31 July 1947) blood samples were again collected from this group and from Group A to whom no additional vaccine was administered.

2. Results - Local and Systemic Reactions - In Group A 14 of 60 soldiers were found to have areas of induration 2 cm. or more in diameter at the site of injection within 24 hours. Approximately the same number showed similar reactions following subsequent injections. No marked erythema was noted. Two men reported headaches. One man spiked a temperature (38.9 C.) developed generalized "aches" and severe malaise during the first 24 hours. Neurological examination was negative and he became clinically normal within 48 hours following the injection. Before subsequent injections he was given 0.5 cc 1:1000 epinephrin and developed no reaction. In Group B 60 of 70 men showed erythema and induration 2 cm. or more in diameter at the site of injection within 24 hours. Approximately the same number showed similar reactions following subsequent intradermal injections. Two of the group reported slight headaches following the first injection, but no febrile reactions were reported. No ill effects were noted in Group C.

Neutralizing Antibody Response: For purpose of vaccine evaluation in a known non-immune population during a period when natural concommitant infection is extremely unlikely the development of one log or more in the neutralizing antibody content of a serum previously found to be devoid of neutralizing antibodies has been arbitrarily taken as a "satisfactory" response. The magnitude of the rise is recorded in all cases to permit regrouping of the data in any way desired to permit comparison with other studies.

When tested after the initial series of three doses had been administered the percentage of sera, which changed to positive from negative was low in both Groups A and B. In Group A 24 (38%) of 65 vaccinated subcutaneously showed a satisfactory response while in Group B only 18 (27%) of 67 persons vaccinated intradermally showed a rise of similar magnitude (See Table 11). When the X^2 test is applied the apparent difference can be due to chance. There was no response noted in Group C.

Following the administration of an additional 2.1 cc. of vaccine to Group B 18 (54.5%) of the 33 remaining members showed a "satisfactory" response in contrast to the previous figure of 27%. No significant change was noted in Group A when tested at the same time, so that the likelihood of inapparent infection is considered negligible. (See Table 12.)

Table 11. Response in Serologically Negative American Adults Following Administration of Japanese B Encephalitis Vaccine (Three doses).

Route of Inoculation	Logs Protection[x] 3.0/	2.0/	1.7[xx]	1.0/	Negative	Total Tested
A. Subcutaneous (3.0 ml.)	5(8%)	17(27%)	19(30%)	24(38%)	39(62%)	63
B. Intradermal (0.3 ml.)	4(6%)	11(16%)	15(22%)	18(27%)	49(73%)	67

[x] Totals are cumulative

[xx] Corresponds to Neutralization Index of 50 or more

Table 12. Response in Serologically Negative American Adults Following Administration of Japanese B Encephalitis Vaccine.

Route of Inoculation	3.0/	2.0/	1.7/[xx]	1.0/	Negative	Total Tested
A. Subcutaneous (3.0 ml.)	2(6%)	7(21%)	9(27%)	11(34%)	21(66%)	32
B. Mixed (0.4 ml. ID 2.0 ml. SC)	2(6%)	11(33%)	16(48%)	18(54%)	15(46%)	33

[x] Totals are cumulative

[xx] Corresponds to Neutralization Index of 50 or more

3. Discussion - A summation of American literature dealing with the development of neutralizing antibodies in human beings following administration of Japanese B encephalitis vaccine has been presented by Warren, et al (62). Koprowski and Cox (56) studied the comparative response elicited by mouse brain and chick embryo type vaccine in 7 individuals. Series reported by Sabin (61) and by Hammon (64) were composed of individuals living in endemic or epidemic areas so that an anamnestic response can not be eliminated. Further, these last two authors, at least in part, were using vaccine of doubtful potency. Warren, et al. (62) believe that their observations support Sabin's data suggesting that the antibody response is directly proportionate to the potency of the vaccine as measured by mouse protection test. The vaccine used in the present study was of acceptable potency.

In the two groups reported herein there was no difference beyond a statistical fortuitous range between the subcutaneous and intradermal routes of vaccine, despite the disparity in total amount of vaccine administered. However, the administration of additional vaccine to Group B definitely elicited further response, suggesting that in addition to potency total size of dose may be of importance.

Although the same lot of vaccine was utilized the vaccination response in Japanese children was far better than when equivalent doses were administered to soldiers. This agrees with results reported by Sabin (61) using mouse brain vaccine so far as the age factor is concerned. It is possible, but considered unlikely, that an anamnestic rise obtained in the Japanese children but a more likely explanation would appear to lie in the ratio of the amount of antigen to body weight.

Warren, et al., report that following 2 doses of 2 cc. each with a 4 day interval 72% (31/43) of the individuals tested failed to exhibit as much as 1 log of protection.

When these same authors (62) administered 3 doses of vaccine following the same schedule as used herein, 19 of 33 individuals showed a "satisfactory" response after subcutaneous administration, while

5 of 9 individuals showed a "satisfactory" response following intracutaneous administration. The results are not significantly different but mention is made that the neutralizing antibody level obtained was definitely higher by the subcutaneous route. No such trend is apparent in the presently reported results, nor do the present neutralization antibody levels approach those observed in clinical cases of the disease except infrequently.

4. Summary - A comparison of the response elicited by Japanese B encephalitis vaccine administered by various routes and schedules to American adults has been presented. In one group vaccinated by a combination of intra-dermal and subcutaneous methods (total of 2.4 ml. in 6 doses over a period of 74 days) was observed the highest response yet obtained in Americans on initial immunization by members of this unit. The numerical response, in no instance, approximates that observed in Japanese children.

VII. Antibody Response in Military Personnel Following Recall Administration Of Japanese B Encephalitis Vaccine

Concurrently with the attempt to evaluate the response elicited by initial administration of vaccine by subcutaneous and intracutaneous routes, a similar attempt was made in 1947 to ascertain the effectiveness of single subcutaneous and intradermal recall doses in American Military Personnel. Studies were continued in 1948.

1. Materials and Methods - a. 1947 - An attempt was made to obtain two additional groups of soldiers similar in all respects to those previously described except that all had received full courses of Japanese B encephalitis vaccine prior to expected seasonal advent in 1946. Approximately 50% of the volunteers in each group had been immunized with 2 doses of 2 ml. each and the remaining 50% with 3 doses of 1 ml. each. Part of the immunization had been accomplished with mouse brain type vaccine and part with chick embryo type. Group R-A received one recall dose of 1.0 ml. of the vaccine (Lot 101-A) subcutaneously (17 April 1947) and group R-B, one of 0.1 ml. (Lot 101-A) intradermally. The volunteers were bled prior to vaccination and 2 weeks following the recall dose of the vaccine to obtain serum for neutralization tests. Ages of the volunteers ranged from 19 to 43 and from 18 to 45 in group R-A and R-B respectively, the means being 28.8 $\pm$ 6.87 and 23.7 $\pm$ 6.82.

Group C had not previously received any Japanese B vaccine. This group was used as control and was injected intradermally with one dose of 0.1 ml. of typhoid vaccine instead of Japanese B vaccine. Blood samples were obtained prior to, and two weeks subsequent to vaccination. The ages of these men were between 17 and 21, the mean being 18.6 $\pm$ 0.06. (This was the same group C noted in the preceding paper).

Blood samples were collected and handled in the manner previously described. Simultaneous testing of pre- and post-vaccination sera was not possible; hence, the results presented constitute a number of different tests.

b. 1948 - In March 1948 an additional number of military personnel (Far East Air Forces) with histories of having received variable amounts of vaccine were tested for complement fixing and neutralizing antibodies prior to and following administration of a single subcutaneous recall dose of 1.0 ml. of Japanese B encephalitis vaccine (AMDR&GS Lot 205-A, ID_{50} - 0.008). Post-vaccination specimens were obtained two weeks after vaccination. All paired specimens were tested simultaneously. Groups included individuals who had received vaccine in 1946 (mouse brain) and 1947 (R-D); those who had received a previous course in 1947 only (R-E); and as controls, a small group with no history of JBE vaccination (F). Immunization registers showed wide variation both in route of administration and in number of doses of vaccine given previously, particularly in 1947.

2. Results - a. 1947 - Local and Systemic Reactions - Following the vaccination, local induration and erythema were frequent, while systemic reactions (fever, headache, malaise, nausea, etc.) were observed in a few persons. Thus 25 of 31 in group R-A were found to have areas of induration 2 cm. or more in diameter at the site of the subcutaneous injection within 24 hours. No marked erythema was noted. Two of the men complained of systemic reactions (headache 1; diffuse transient muscle cramps 1). One febrile reaction (37.3° C.) was reported. Of group R-B, 27 in number, 17 showed erythema and induration 2 cm. or more in diameter at the site of the intradermal injections, within 24 hours. None of this group had axillary adenopathy. Two of the total group complained of nausea during the first 24 hours following the vaccination. One febrile reaction (37.3° C.) was reported.

Neutralizing Antibody Response - For purposes of vaccine evaluation during a period when natural concommitant infection is extremely unlikely the development of one log or more in the neutralizing antibody content of a serum previously found to be devoid of neutralizing antibodies has been arbitrarily taken as a "satisfactory" response. The magnitude of the rise is recorded in all cases to permit regrouping of the data in any way desired to permit comparison with other studies.

Table 13. Response in Previously Vaccinated but Serologically Negative American Adults Following Administration of Single Recall Dose Japanese B Encephalitis Vaccine - 1947

Route of Inoculation Group	Logs Protection[x] 3.0/	2.0/	1.7/[xx]	1.0/	Negative	Total Tested
R-A 1.0 ml. SC	4(15%)	11(41%)	13(48%)	13(48%)	14(52%)	27
R-B 0.1 ml. ID	2(4%)	8(32%)	11(42%)	11(42%)	15(58%)	26
C. Control		Negative			75	75

[x] Totals are cumulative

[xx] Corresponds to Neutralization Index of 50

Table 14. Response in Previously Vaccinated American Adults Following Administration of Single Recall Dose of Japanese B Encephalitis Vaccine - 1948

Route of Inoculation Group	Complement Fixation Positive	Logs Protection[x] 3.0/	2.0/	1.7/[xx]	1.0/	Negative	Total Tested
R-D (1946&1947) Pre-vaccination	0	0	3(18%)	3(18%)	5(29%)	12(71%)	17
R-D (1946&1947) Post-vaccination	0	5(38%)	6(46%)	7(54%)	10(77%)	3(23%)	13
R-E (1947) Pre-vaccination	0	0	5(14%)	5(14%)	11(30%)	25(70%)	36
R-E (1947) Post-vaccination	1/34(3%)	11(37%)	18(60%)	19(63%)	24(80%)	6(20%)	30
Total of R-D and R-E Pre-vaccination	0	0	8(15%)	8(15%)	16(30%)	37(70%)	53
Total of R-D and R-E Post-vaccination	1/45(2%)	16(37%)	24(56%)	26(60%)	34(79%)	9(21%)	43
F (Control) Pre-vaccination	0	0		0	0	8	8
F (Control) Post-vaccination	0	0		0	1(17%)	5(83%)	6

[x] Totals are cumulative

[xx] Corresponds to Neutralization Index of 50 or more

The results of the recall doses are summarized in Table 13. Persons (two in group R-A and two in group R-B) whose pre-vaccination specimens were positive were omitted. Group C was included as a control to check the possibility of inapparent infection. Pre- and post-vaccination (typhoid) sera of 75 volunteers in group C were all negative for neutralizing antibodies against Japanese B virus. The response of the two groups was quite similar. In the subcutaneous group, 13 out of 27 volunteers (48%) showed a response following vaccination, while in the intradermal group 11 out of 26 persons (42%) showed a similar response. The slight difference in percentages of positives was not significant when calculated X^2.

b. 1948 - Serologic Response - None of 18 individuals vaccinated in 1946 and 1947 (average of 4.3 previous doses per man) showed positive complement fixing antibodies for JBE virus in March 1948, nor did any of 39 individuals previously inoculated in 1947 (average 3 previous doses per man). None of 11 subjects in the first group and only one of 34 in the second group showed a positive reaction (4 / at 1:4) 14 days after administration of the 1 ml. subcutaneous recall dose.

The percentage of individuals showing positive neutralization indexes in the two groups in pre-vaccination (1948) specimens did not appear to be significantly different. 3 of 17 (18 percent) in group R-D and 5 of 36 (14 per cent) in group R-E fell in this category. 70 per cent of each group had negative neutralization indexes, with the remaining individuals showing indexes in the equivocal range. Following administration of the recall dose, 54 percent (7/13) of the first group and 63 per cent (19/30) of the second group showed positive neutralization indexes, with 23 percent and 20 per cent of the two groups, respectively, remaining negative.

In contrast, none of the small group (F) of previously non-vaccinated individuals showed positive complement fixation reactions or neutralization indexes either before or after receiving a single dose of potent vaccine. One of 6 changed from a negative to an equivocal neutralization index. Results are summarized in Table 14.

3. Discussion - a. 1947 - Warren et al. (62) reported that five of eight individuals, who had previously received a full course of Japanese B encephalitis vaccine, developed neutralizing antibodies following 1.0 ml. of lyophilized chick embryo vaccine (ID_{50} of 0.011 ml.). In contrast they noted that eight of eight similar volunteers gave a "satisfactory" response following administration of 0.1 ml. of lyophilized chick embryo vaccine (ID_{50} of 0.015 ml.) Further, the mean index of neutralizing antibodies was higher in this last group than in the first group vaccinated by the subcutaneous route.

No such apparent difference in response was observed in the groups recorded here. Certain differences in the two studies are worthy of comment. Warren's total of 16 individuals contained 9 who had shown a response following initial vaccination in 1946. Eight of these same 9 showed a measurable antibody level prior to administration of the recall dose. In the present series only 4 of 57 subjects had any serologic evidence of previous vaccination prior to administration of the recall dose. Although not included in the tabulation, each of these four men showed an added response following vaccination. On this basis the groups are non-comparable, either as a result of inclusion of a large number of "non-reactors" or because the vaccine administration under field conditions elicited a poorer original response than obtained in the group studied by Warren,et al. Because of the response of almost 50% following the recall dose the latter explanation seems most applicable. The possibility that Warren's group may have exhibited an enhanced response due to multiple vaccination with chick embryo vaccines of various types must also be considered. It may be that the response to dual or linked-antigens (virus proteins and chick-embryo proteins) in sensitized or "hyper-immune" individuals may vary according to the method of administration.

Previous studies have shown that a statistically non-significant difference in neutralizing antibody response exists between the two methods of administration of chick embryo vaccine as a recall dose in Japanese children. A similar condition appears to exist in this instance, but again slightly better response to the larger subcutaneous dose is noted.

b. 1948 - So far as can be ascertained there is little chance that any of the individuals comprising the 1948 group had any chance to have experienced contact with the virus of Japanese B encephalitis under natural conditions. Consequently, it is considered that the responses elicited are due to the vaccination alone.

There is apparently no difference between Groups R-D and R-E and because of variations in the potency, methods of administration, and total number of doses of vaccine given it is not believed that comparison is justified.

The responses obtained in 1948 are somewhat higher than those noted in 1947 but since the 1948 group contains individuals with measurable amounts of neutralizing antibodies prior to the recall vaccine the difference is less than might appear. However, approximately 30 percent of individuals originally negative prior to the 1948 recall dose failed to develop any response to the vaccine in contrast to 52 per cent in the 1947 series.

The recall response obtained in 1948 was definitely higher than any available figures on the response to initial vaccination. It remains lower in total response and magnitude of response than obtained on initial administration of vaccine to Japanese children (Section II) and definitely lower than the response of Japanese children to a comparable number of recall doses (Section IV). As has been noted earlier, the possibility of an anamnestic factor operating in the latter group cannot be wholly eliminated.

4. Summary - In 1947 chick embryo Japanese B encephalitis vaccine elicited a measurable response when administered by intracutaneous and subcutaneous routes as a recall dose to previously vaccinated but serologically negative American Military personnel in approximately 50% of each group. No obvious difference between methods is apparent.

In 1948 in a study using only the subcutaneous route a measurable response was elicited in approximately 80 percent. No difference could be demonstrated between groups in which this was the initial recall dose and in which this was the second recall dose.

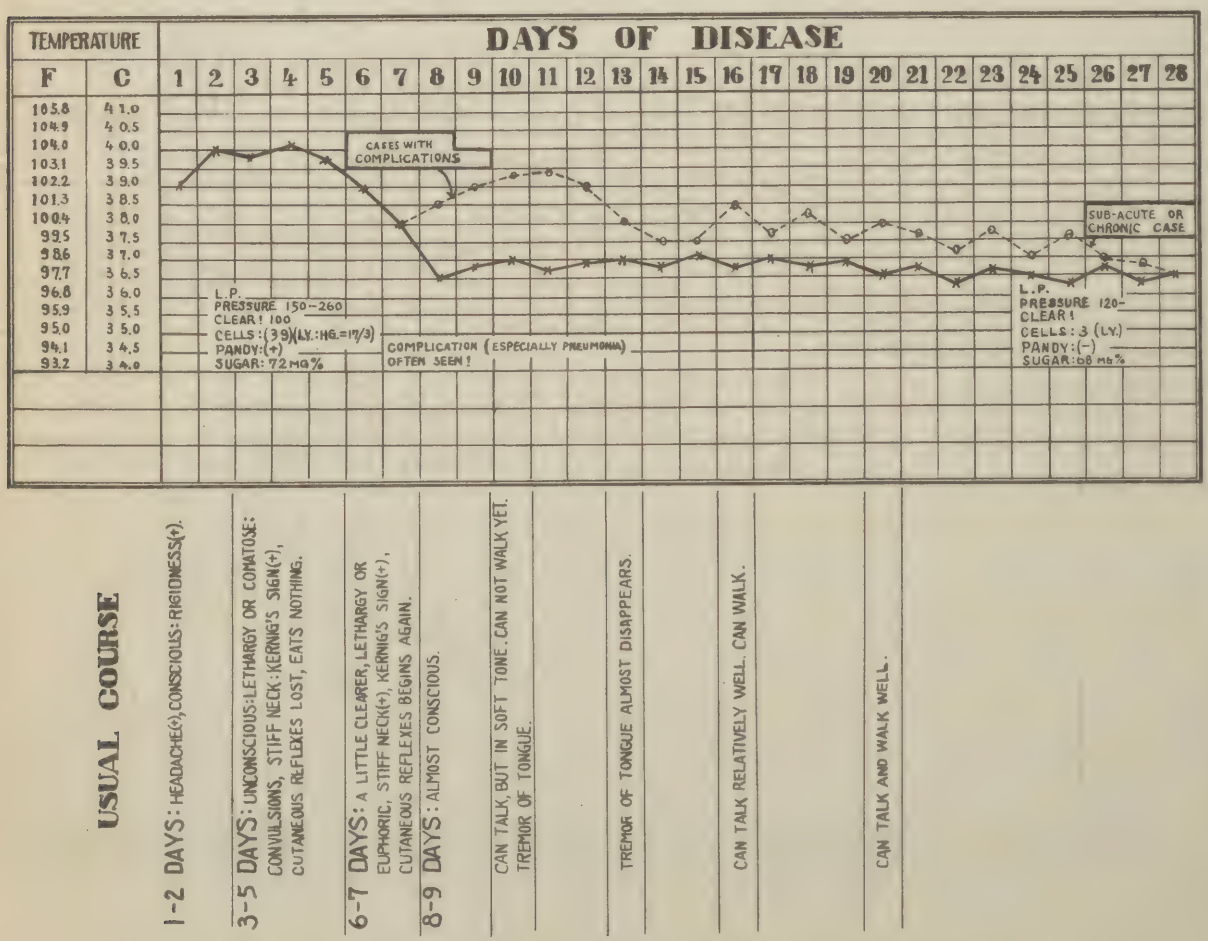
VIII. Certain Epidemiological Aspects of Japanese B Encephalitis in 1948

During 1948 approximately 8,000 cases of "summer" encephalitis were reported in Japanese nationals. More than one-quarter of these cases occurred in the Tokyo area where the disease was initially recognized. The remainder of the cases were scattered throughout the main islands of Japan, including Hokkaido. During the same period an epizootic of 3,697 cases occurred in equines. Cases of equine disease were observed in the Tokyo area several days prior to the recognition of human cases.

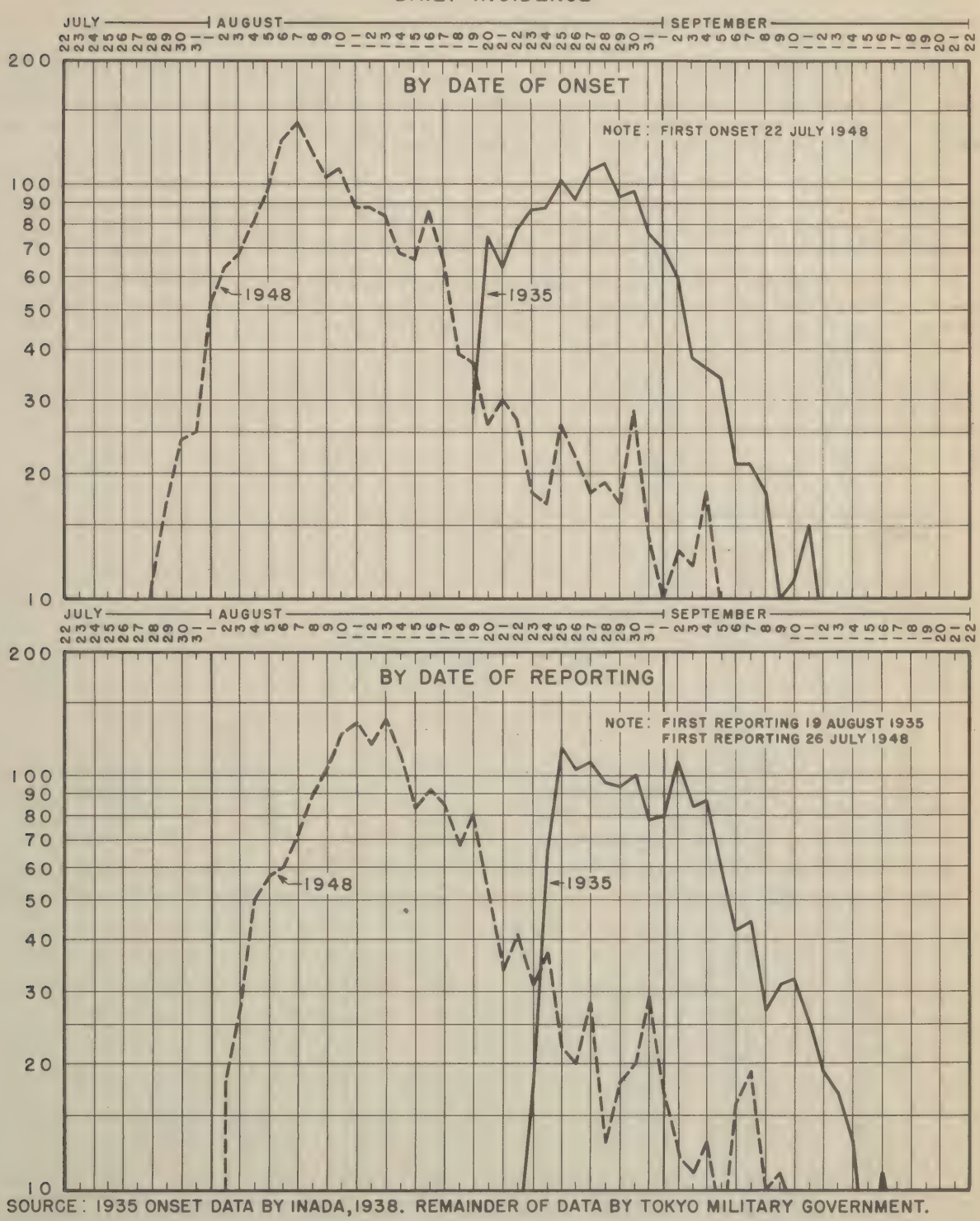
This particular discussion does not presume to be complete and reference will be made only to certain aspects of the disease, intended as a background for various epidemiological considerations. Other phases will receive only brief mention since they were recorded on film for a more adequate presentation. Information is fairly complete on the cases occurring in Tokyo and these will be emphasized.

1. Clinical Aspects - Experienced American observers saw many of the cases in the Tokyo area. Others of these cases were seen by experienced Japanese physicians and the clinical picture, when the disease occurs in epidemic form, is fairly typical. In 1925 Kaneko (71) wrote of the 1924 Okayama epidemic and his clinical description would well apply to the cases seen in Tokyo and elsewhere in Japan in 1948. Similarly, the clinical aspects of Okinawa cases of 1945, well presented in recent American literature by Sabin (23) and by Lewis, et al. (72) depict, with minor differences, the same picture observed in Japan. Taylor (10) has recently presented a resume of the 1948 Japanese cases. Figure 4, a composite of these cases, is reproduced from his presentation.

Figure 4. Composite of 475 Japanese B Encephalitis Cases - Komagome Hospital, Tokyo, 1948



JAPANESE B ENCEPHALITIS — TOKYO-TO



SOURCE: 1935 ONSET DATA BY INADA, 1938. REMAINDER OF DATA BY TOKYO MILITARY GOVERNMENT.

FIG. 5a

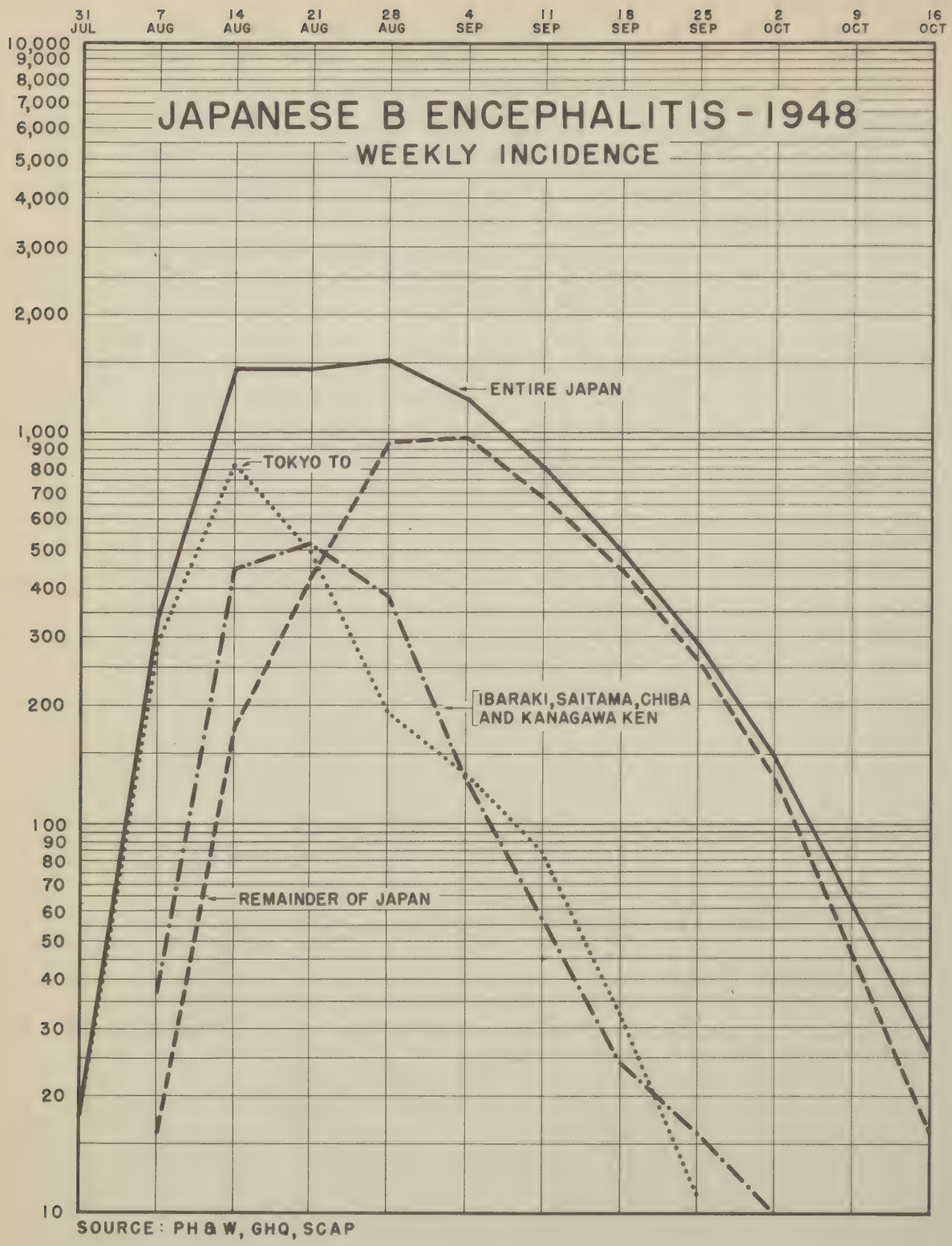

JAPANESE B ENCEPHALITIS - 1948
WEEKLY INCIDENCE
31 JUL
7 AUG
14 AUG
21 AUG
28 AUG
4 SEP
11 SEP
18 SEP
25 SEP
2 OCT
9 OCT
16 OCT
10,000
9,000
8,000
7,000
6,000
5,000
4,000
3,000
2,000
1,000
900
800
700
600
500
400
300
200
100
90
80
70
60
50
40
30
20
10
ENTIRE JAPAN
TOKYO TO
IBARAKI, SAITAMA, CHIBA AND KANAGAWA KEN
REMAINDER OF JAPAN
SOURCE: PH & W, GHQ, SCAP

FIG. 5b

JAPANESE B ENCEPHALITIS

BY PREFECTURE

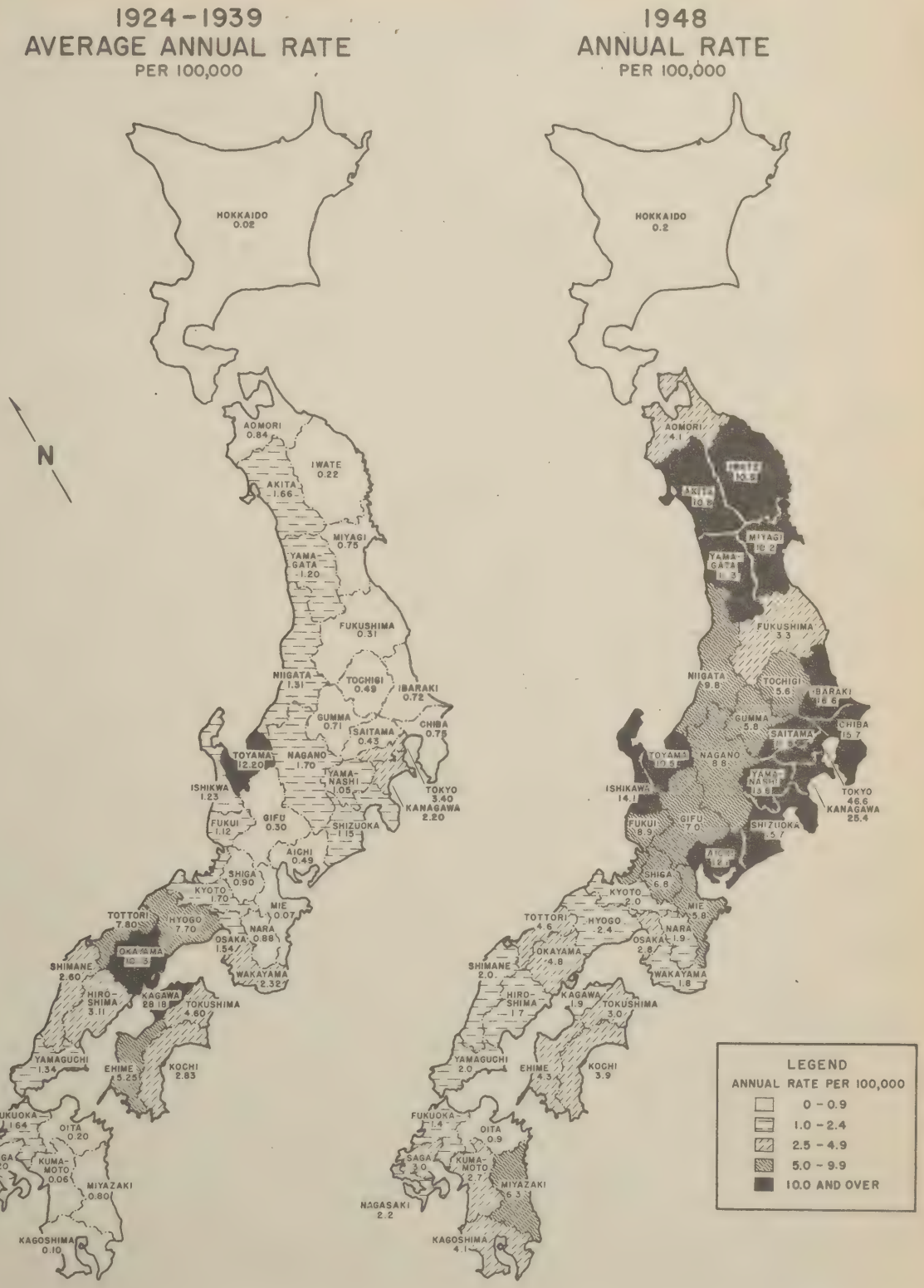


FIG. 6

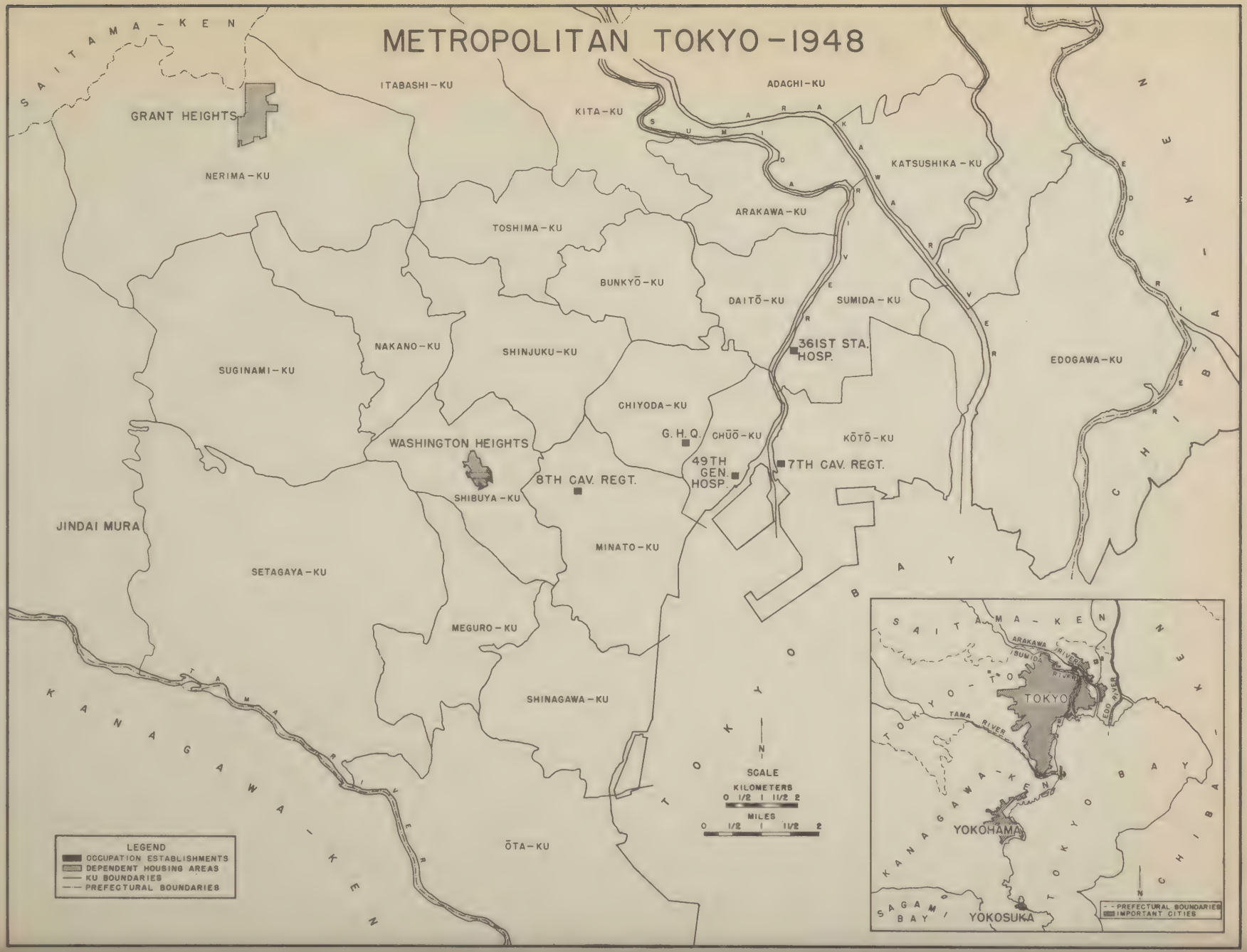

METROPOLITAN TOKYO - 1948
SAITAMA-KEN
GRANT HEIGHTS
ITABASHI-KU
KITA-KU
ADACHI-KU
NERIMA-KU
SUMIDA RIVER
KATSUSHIKA-KU
ARAKAWA-KU
TOSHIMA-KU
ARAKAWA RIVER
NAKANO-KU
BUNKYŌ-KU
DAITŌ-KU
SUMIDA-KU
SHINJUKU-KU
361ST STA. HOSP.
EDOGAWA-KU
SUGINAMI-KU
CHIYODA-KU
G. H. Q.
CHŪŌ-KU
KŌTŌ-KU
WASHINGTON HEIGHTS
49TH GEN. HOSP.
7TH CAV. REGT.
8TH CAV. REGT.
SHIBUYA-KU
JINDAI MURA
MINATO-KU
SETAGAYA-KU
MEGURO-KU
SHINAGAWA-KU
TOKYO BAY
KANAGAWA-KEN
TAMA RIVER
ŌTA-KU
N
SCALE
KILOMETERS
0 1/2 1 1/2 2
MILES
0 1/2 1 1/2 2
LEGEND
OCCUPATION ESTABLISHMENTS
DEPENDENT HOUSING AREAS
KU BOUNDARIES
PREFECTURAL BOUNDARIES
TOKYO
YOKOHAMA
YOKOSUKA
SAGAMI BAY
PREFECTURAL BOUNDARIES
IMPORTANT CITIES

FIG 7

Table 15. Reported Cases of Japanese B Encephalitis in Tokyo by Age Group and Location
Source - Tokyo Military Government Public Health and Welfare Section
1948

Population - 1 Aug. 1948

Cases - 1 November 1948

Location	Age Group 1-5	6-10	11-20	21-30	31-40	41-50	51-60	31-60	60 --	Total	Rate	1935 Total	1935 Rate
Chiyoda	9/9,092	7/8,218	6/18,266	8	2	1	2	13/58,414	3/4,725	38/98,715	38.5	62/197,233	31.5
Chuo	13/14,369	15/13,182	7/23,104	11	3	3	2	19/86,844	2/7,936	56/150,436	37.2	60/261,205	23.1
Minato	17/18,054	15/16,703	16/33,302	11	2	3	2	18/105,047	3/10,082	69/183,188	37.6	142/337,333	42.2
Shinjuku	20/17,684	21/16,746	10/32,318	8	2	1	1	12/99,676	3/9,466	66/175,890	37.5	103/375,848	28.8
Bunkyo	14/15,288	23/14,525	15/29,606	4	2	1	5	12/89,744	1/9,485	65/158,649	40.9	71/288,440	24.6
Daito	35/22,757	40/21,456	17/42,330	11	3	2	3	19/122,345	4/11,437	115/220,325	52.2	117/464,217	25.2
Sumida	16/19,853	17/19,626	22/26,525	5	2	0	2	9/96,511	1/8,091	65/180,606	36.0	76/464,892	16.9
Koto	22/13,863	31/12,644	22/21,938	9	3	3	1	16/64,934	0/4,726	91/118,105	77.0	74/385,212	28.7
Shinagawa	25/25,813	30/23,797	22/45,312	4	2	1	3	10/133,742	3/11,518	90/240,182	37.4	145/366,125	39.6
Ohta	45/38,326	59/35,969	44/62,065	9	8	6	5	28/186,499	5/16,861	181/339,720	52.3	98/348,941	28.4
Setagaya	34/40,731	49/38,669	36/70,093	12	5	4	4	25/202,185	7/21,487	151/373,165	40.5	36/210,701	17.2
Meguro	13/19,388	26/17,875	5/34,129	8	5	2	0	15/100,501	7/9,165	66/181,050	36.5	48/266,928	16.2
Shibuya	22/14,703	24/13,760	9/26,672	4	1	3	1	9/83,614	5/8,044	69/146,793	47.0	92/238,850	35.9
Nakano	9/10,918	13/17,689	5/33,792	2	2	3	1	8/99,968	4/9,464	39/178,831	20.9	70/176,383	39.5
Suginami	15/31,445	14/28,997	17/53,893	5	7	2	3	17/166,226	8/17,491	71/298,052	23.8	48/109,217	43.9
Toshima	20/18,122	18/17,271	15/32,272	6	1	0	2	9/92,767	0/8,324	62/168,756	36.7	76/268,015	28.6
Arakawa	23/18,100	40/17,835	22/33,810	11	3	1	1	16/85,398	2/7,561	103/162,734	63.3	65/326,210	28.4
Adachi	49/29,691	28/28,725	41/47,578	11	7	1	5	24/122,097	3/12,800	145/240,891	60.2	38/174,612	21.9
Kita	17/24,618	40/23,587	23/43,127	13	1	0	1	15/118,789	0/10,681	101/220,802	45.0	43/285,561	14.9
Itabashi	27/23,929	25/22,100	9/35,424	10	4	2	1	17/98,875	1/9,163	79/189,491	41.7	29/150,868	19.3
Nerima	3/12,796	16/12,482	5/22,631	5	1	0	1	7/59,227	2/6,547	33/113,683	29.0	--	--
Katsushika	11/27,805	19/26,053	11/43,820	4	4	0	2	10/116,325	1/11,026	52/225,029	24.4	11/105,682	10.5
Edogawa	7/22,861	30/22,437	22/37,495	8	3	0	4	15/96,544	2/10,127	76/189,464	40.1	21/129,230	15.9
Kitatama	29/41,152	42/37,889	22/63,989	11	5	1	0	17/177,401	7/19,195	117/339,626	34.4	29/292,929	9.9
Minamitama	6/17,007	13/17,323	11/29,360	1	1	0	0	2/66,478	0/10,572	32/140,740	22.7	6/90,909	6.6
Nishitama	5/16,823	4/17,687	4/30,812	1	1	0	0	2/73,599	0/11,384	15/150,305	10.0	1/101,121	1.1
Others[x]	3/4,794	4/4,994	6/8,862	3	3	0	0	6/17,601	2/3,842	21/40,094	--	--	--
Total	509/600,394	669/570,245	444/1,035,740	195	83	40	52	370/2,920,423	76/291,069	2,068/5,417,871	38.17	1,462/6,379,841	22.9

[x] Includes 3 cases from Izu Islands. Remainder of cases in transients in Tokyo. Population figures are of Izu Islands only.

2. Case Incidence - Much of the data on which this section is based were derived directly from reports prepared by the Public Health and Welfare Section of SCAP, and from reports prepared by the Public Health and Welfare Section (39) of Tokyo Military Government. These are reported figures based in many instances on clinical opinions.

The first reported case in Tokyo had an onset on or about 20 July. The outbreak was truly "explosive" reaching a daily peak (onset date) of 137 cases on 7 August and declining thereafter. Practically no new cases were reported after 1 September. The daily incidence is depicted in Figure 5, which also shows similar data for the 1935 epidemic. The 1948 onset was about three weeks earlier than the 1935 onset and an essentially similar relationship exists for the maximum daily reported incidence.

Although earlier epidemics have been reported (37) to begin in the southern portion of Japan such was not true in 1948. Following recognition of cases in Tokyo there were reports from the adjacent prefectures of the Kanto region in 7-10 days. During the month of August cases were reported from almost all of the other prefectures of Japan reaching a total of 7,666. The reported annual incidence (as of 31 December 1948) is shown in Figure 6, as is the reported annual average incidence for the years 1924-1939.

The overall reported weekly incidence for Japan is presented in Figure 5, where an attempt is made to analyze the various components comprising the whole. The overall slightly skewed incidence curve, appears definitely related to the variations in the peaks of the individual components while the sharp initial rise is known to be due at least in part to delay in recognition of cases.

3. Tokyo Case Incidence - In Figure 7 the city plan of Tokyo is presented. The earliest cases were presented in the either partially or completely bombed areas of Kita-ku, Sugnami-ku, and Daito-ku. Detailed data relative to incidence and population are shown in Table 15. More than one case from a family group or a household was extremely rare.

4. Age Incidence of Cases in Tokyo - It will be noted in Table 15 that the population per age group (Japanese age reckoning) in Tokyo is sufficiently homogenous to permit direct comparisons of age specific rates. Examples for certain sections of Tokyo are given in Table 16. Reference to the specific sections chosen will be made in connection with the post-epidemic survey to be presented later. These examples are included here simple to indicate that some of the variations may be the functions of small numbers, and that all such computations may be weighed heavily by human factors.

Table 16. Case Rates (per 100,000) in Certain Areas of Tokyo-ken in 1948

Kus Ages	1-5	6-10	11-20	21-30	31-60	60/	Total	Total 1935
Chuo	90.4	113.8	30.3	37.2	13.9	25.2	37.2	23.1
Daito	153.7	186.4	40.1	26.4	9.9	35.0	52.2	25.2
Ohta	117.4	164.0	70.9	14.2	15.4	29.6	52.3	28.4
Suginami	47.7	48.3	31.5	8.8	10.9	45.7	23.8	43.9
Kitatama	70.5	110.8	34.4	18.2	5.1	36.5	34.4	9.9
	x	x	x	x	x	x	x	
All Tokyo	84.7	117.3	42.9	19.6	9.1	26.1	38.2	26.4

These are neither the highest nor lowest rates. For example the rate in Kita-ku for 1-5 year group is 165, while the rate in Shinagawa-ku for the 6-10 group is 245. (If it is desired to further break down the age incidence calculations may be based on 21-60 age groups as follows: Of the total population for this age group calculate 34 percent between the ages of 21-30, 25 percent for the age groups 31-40, 23 percent for the age group 41-50, and 17 percent for the 51-60 age group).

The overall crude case distribution (and age specific age distribution) is very similar to that reported for the 1935 epidemic in Tokyo (5). These two metropolitan outbreaks differ significantly in age distribution from that reported throughout Japan for the period 1924-1933. During these years the case incidence was much higher in the group over 50 years of age, the difference becoming much more

apparent when calculated on an age specific basis. These earlier figures are heavily weighed by the Okayama epidemic of 1924 but studies of the annual incidence of cases during this period in areas where significant numbers of cases occurred indicate that this trend (predominance of cases in elderly people) was repeated. While cases of apoplexy, senility, etc., probably have been erroneously included in the earlier reports it is difficult to believe that all diagnoses of encephalitis in the older age groups could have been in error.

5. Sex Incidence of Cases in Tokyo - In Table 17 the Tokyo cases have been broken down to show the incidence and fatalities in males and females by age groups.

Table 17. Age and Sex of Japanese B Encephalitis in Japanese Nationals - Tokyo 1948

	1-5	6-10	11-15	16-20	21-30	31-40	41-50	51-60	61-70	71/	Total
Cases											
Males	308	404	176	86	99	40	19	28	18	12	1190
Females	201	264	108	74	96	43	21	24	27	19	877
Total	509	668	284	160	195	83	40	52	45	31	2067
Male Cases per 100 Female Cases	153	153	163	116	103	93	90	117	67	63	136
Deaths											
Males	62	63	34	17	23	7	5	10	10	5	236
Females	58	74	27	12	41	18	10	12	21	19	292
Total	120	137	61	29	64	25	15	22	31	24	528
Case Fatality percentage											
Males	20	15	19	20	24	17	25	36	55	42	19.9
Female	29	28	25	16	40	42	50	50	78	100	33.3

For purposes of evaluation of the data presented in the various tables the following male-female ratios on the basis of ration returns existed in Japan in 1948:

	Male	Female
1-5	50.8	49.2
6-10	50.5	49.5
11-15	50.4	49.6
16-20	50.3	49.7
21-25	49.2	50.8
26-60	48.2	51.1
61/	44.1	55.9

(In the 26-30 year group there is a more pronounced deficit of males due to wartime loss)

As has been reported in previous epidemics of Japanese B encephalitis (5, 37) males show the highest case incidence. The magnitude of the difference definitely decreases with the 16-20 age group and tends to disappear thereafter with females showing a slight predominance except in the 51-60 age group. The case mortality rates are higher throughout in females with a definitely increasing mortality above the age of 21 years. The case mortality rate for males seems to remain about the same for the first five decades and then to show a moderate rise.

Careful inquiry has thus far failed to reveal any significant racial practice (such as more interest in male children) that would account for these differences in the reported case incidence. Inspection of data pertaining to the St. Louis epidemics of 1933 and 1937 (Tables 19 and 20, Reference 7) indicates that a similar distribution occurred, with a predominance of males in the ages below 14 years. It is of further interest to note that in the older age groups male cases predominated in the 50-59 age group of the 1933 and 1937 St. Louis epidemics.

6. Mortality - Certain mortality characteristics of the Tokyo epidemic are shown in Table 17. The Tokyo case fatality rate was 25.7 while the extremes reported from the various sections of the Tokyo area were 15.3 (Itabashi-ku) and 35.7 (Chuo-ku). (Of the wards listed in Table 16 the case fatality rates are: Chuo-ku 35.7; Daito-ku 27.1; Ohta-ku 25.4; Suginami-ku 26.9; Kita-tama 26.4). For all of Japan the case fatality rate is somewhat higher, approximately 37.0. These figures are still subject to change due to delayed corrections. Such rates are definitely lower than those previously reported in Japan (5, 37) and it is believed that the change is due, at least in part, to better recognition of the milder cases.

7. Serologic Diagnosis - During 1948 approximately 5,000 serum specimens were received and processed from Japanese suspect cases from 42 prefectures. Complement-fixation tests have not yet been completed. At this time positive tests number 404 instances from 1,013 suspect cases. Of 234 Tokyo cases positive results have been obtained in 147 instances. Many of the sera were improperly stored during transmission and in many the time of sampling was not optimal. Consequently, it is believed that these figures actually bespeak a much higher incidence of disease than might appear. Due to variations in the time intervals between the initial specimen and the follow-up specimens and to other irregularities (such as whether the onset date was given for prodromal symptoms or for development of neurological symptoms) it is difficult to present a true chronologic picture of the time of development of complement fixing antibodies. As a composite impression it would appear that at least one half of the indigenous cases became serologically positive by the eleventh day and that 80 percent were positive by the fifteenth day. A few remained negative until much later and a few positives were reported on the third day of disease.

8. Virus Isolations - During the epidemic virus strains were isolated from 12 fatal cases, using brain tissue in each instance. In most of these instances, and in several cases in which original attempts were unsuccessful here, brain tissue preserved in glycerine-saline have been forwarded to AMDR&GS.

Isolation procedures utilized have been previously described. Symptoms were noted in first animals in all instances. Identification of these agents by means of serological reactions indicates that a similarity exists in antigenic and immunogenic structure between the virus of Japanese B encephalitis (Nakayama strain) and all of the above isolates. These serologic reactions are summarized in Table 18. The results given under the heading of Neutralization Index represent the degree of protection afforded animals challenged with the isolate by known specific sera for Japanese B encephalitis, St. Louis encephalitis, and Western equine encephalitis. Results given under the Complement Fixation heading represent titers obtained when the isolate was used as an antigen with known specific hyperimmune sera. Antigens for complement fixation were prepared from isolate material, usually in the 7th successive mouse passage by emulsifying the brain tissue into a 10^{-1} suspension. This was followed by centrifugation, and repeated freezing and thawing in lusteroid tubes suspended in ethanol and solid carbon dioxide until precipitation occurred. The supernatant acted as the antigen.

9. Climate - The importance of climatic conditions on the occurrence of disease in which mosquito transmission is a consideration is obvious. Figure 8 depicts the weather conditions prevailing in Tokyo in 1948 (10).

10. Control Measures - Turner (10) has described the control measures adopted in Tokyo during the 1948 season. For various reasons the actual control program did not begin until after the epidemic was recognized. These measures consisted "of adult control by interior residual spraying of habitations with DDT immediately after each case is reported. An area of not less than 50 meters surrounding the house is also sprayed. Then a larvae control team follows to prevent development of additional adult mosquitoes in the area surrounding the house." (10) Practically no information is available concerning mosquito incidence in Tokyo either before or during the epidemic.

(Note: Japanese workers (M. Kitaoka, et al) have reported that during the summer of 1948, from mosquitoes caught in Tokyo, virus isolations from a pool of Culex tritaeniorhynchus, a pool of mixed Culex species, and a pool of Anopheles hyrcanus sinensis Wiedmann were made.)

Table 18. Neurotropic Isolates (Human - Japanese) Tokyo 1948

No.	Name	Address	LD 50	Neut. Index JBE	Neut. Index SLE	Neut. Index WEE	C.F. Titre JBE	C.F. Titre SLE
V-3213	Fujita, Aiko	Komagome Hosp.	7.8	1000	1.0	1.0	0	1:2
V-3236	Hashimoto, Aiko	Komagome Hosp.	8.5	16000	1.0	1.0	1:32	1:4
V-3632	Koito	Komagome Hosp.	8.2	79000	25	10	0	0
V-3635	Ueoka	Komagome Hosp.	8.5	1000	4.0	1.0	1:32	1:4
V-3636	Shimizu	Komagome Hosp.	6.6	13000	10	1.0	1:32	0
V-3637	Miyake	Komagome Hosp.	6.9	1000	1.0	1.0	1:8	0
V-3639	Ito	Komagome Hosp.	7.8	79000	100	1.0	1:4	1:2
V-3952	Yoshida, Hideo	1st Natl Hosp.	6.4	2500	1.3	1.0	1:32	0
V-3640	Kawamura	Komagome Hosp.	7.9	7900	1.0	1.0	1:8	0
V-3953	Shiraishi, Kuniko	1st Natl. Hosp.	8.5	7900	1.0	1.0	1:16	0
V-4304	Watanabe, C.	Komagome Hosp.	7.5	7900	1.0	2.0	1:16	1:2
V-4305	Hayashi, C.	Komagome Hosp.	5.4	10000	1.0	1.0	1:16	0

Figure 8.

HUMIDITY, TEMPERATURE AND RAINFALL IN TOKYO

MAY - AUGUST 1948

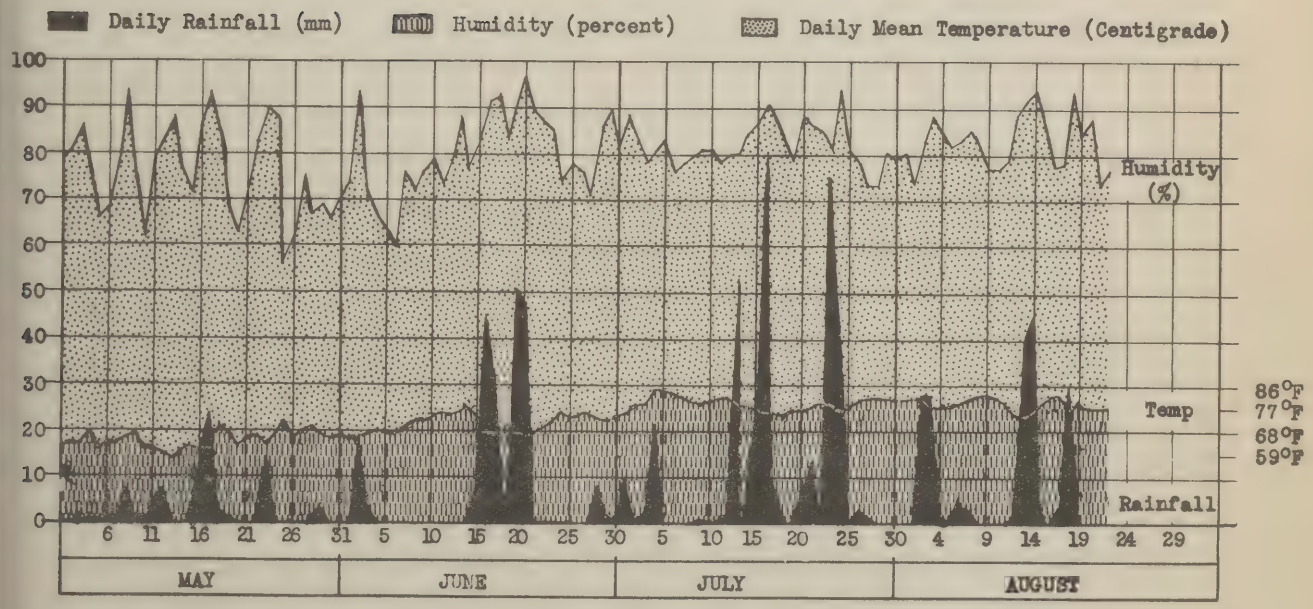


Table 19. Summation of American Cases

No.	Name	Age	Race	Sex	Rank	Location	JBE Immunization 1946	1947	1948	Category	Date of Onset	Date of Adm.	Symptoms	Disposition
1	Ballard, E.	32	C	M	Pvt	Okinawa 574 TC	0	0	3 (25 May)	A	2 Aug	3 Aug	Mild	Duty
2	Baugher, Thomas	8	W	M	Civ. Dep.	Tokyo GHQ	0	3	0	C	8 Aug	10 Aug	Severe	Death
3	Bertram, Kenneth	23	W	M	Pfc	Yokohama 138 AAA Bn	0	3	7 July	A	23 Aug	27 Aug	Mild	Duty
4	Bingham, R.	30	W	M	Cpl	Tokyo 2nd Cav Br	0	0	0	D	9 Aug	11 Aug	Moderate	Duty
5	Bromley, James	28	W	M	Sgt	Yokohama 163rd Tr Car	0	0	3	A	4 Aug	6 Aug	Mild	Duty
6	Burroughs, M.D.	23	W	M	Navy	Yokosuka Navy	0	0	0	D	28 Aug	28 Aug	Severe	Duty
7	Carr, David E.	29	W	M	Sgt	Yokohama 753rd AAA	0	3	7 July	A	5 Aug	15 Aug	Moderate	Duty
8	Corbitt, James	21	W	M	Pfc	Tokyo GHQ	0	0	3 (9 July)	A	18 Aug	28 Aug	Mild	Duty
9	Dawson, Delmar	18	W	M	Rct	Tokyo 5th Cav Reg	0	0	28 Jul 12 Aug	C	14 Aug	21 Aug	Mild	Duty
10	D'Agostino, A.	26	W	M	Cpl	Tokyo 71st S S Bn	0	3	30 Jun	A	19 Aug	23 Aug	Moderate	Duty
11	D'Entremont, G.	40	W	F	Civ. Dep.	Tokyo 584th En Bn	0	0	3 Aug	D	6 Aug	8 Aug	Severe	Evac.
12	Dixon, Robert	21	C	M	Pfc	Otawa 24th Inf Reg	0	0	3 (9 Jul)	A	20 Aug	21 Aug	Moderate	Duty
13	Goodman, W.	30	W	M	Pvt	Tachikawa 370th MVS	0	0	11 Jun	C	21 Aug	23 Aug	Severe	Duty
14	Gorman, Leo	18	W	M	Pvt	Kobe 594th OSC	0	0	2 Jul 24 Aug	C	24 Aug	28 Aug	Moderate	Duty
15	Gosselin, F. R.	20	W	M	Navy	Navy Chipola	0	0	0	D	21 Aug	21 Aug	Moderate	Duty
16	Hardy, Russel T.	26	W	M	Cpl	Tachikawa 808th Eng AB	0	0	0	D	4 Aug	10 Aug	Moderate	
17	Hill, T.L.	25	W	M	Navy	Yokosuka Navy	0	0	3 12 Jul	A	7 Aug	9 Aug	Moderate	Duty
18	Howard, Gordon	24	W	M	1st Lt	Tokyo 8230th Sv Det	0	3	2 Jul	A	5 Aug	7 Aug	Mild	Duty
19	Laggan, Paul	25	W	M	Pvt	Shiroi AB 611th AC&WS	0	3	30 Jun	A	26 Aug	2 Sep	Severe	Duty
20	McCollum, John	19	W	M	Pfc	Tachikawa 987th AES	0	3	0	C	30 Jul	2 Aug	Severe	Duty
21	Moore, Lester	21	C	M	T/5	Okinawa 624th Port Co.	0	0	3 (25 May)	A	25 Jul	27 Jul	Severe	Death
22	Myers, Tom	17	W	M	Civ. Dep.	Tokyo CID, GHQ, FEC	0	0	3 (12 Aug)	C	12 Aug	17 Aug	Mild	Recovery
23	Ozment, Steve	30	W	M	Pfc	Tokyo 1st Med Sq	0	0	0	D	4 Aug	9 Aug	Severe	Death
24	Powell, W.E.	24	W	M	Navy	Yokosuka Navy	2	2	14 Jul	A	15 Aug	17 Aug	Severe	Duty
25	Quinn, Robert	23	W	M	Pfc	Tokyo 8th Cav Reg	0	2	1	B	10 Aug	12 Aug	Moderate	Duty
26	Schmidt, Jos.	27	W	M	Navy	Yokosuka Navy	0	0	2	B	14 Aug	20 Aug	Moderate	Duty
27	Smith, Phyllis	22	W	F	DAC	Tokyo	0	0	3 (19 Jul)	A	13 Aug	16 Aug	Moderate	Duty
28	Walker, Marion	22	W	F	Civ. Dep.	Yokosuka Navy	0	0	0	D	16 Aug	19 Aug	Severe	Recovery
29	Wellborn, J.M.	24	W	M	Sgt	Tokyo 7th Cav Regt	0	0	0	D	5 Aug	5 Aug	Severe	Death
30	Willis, Hosea	32	C	M	Pvt	Yokohama 76th AAA Bn	0	3	8 Jul	A	11 Aug	13 Aug	Moderate	Duty
31	Wright, G.	41	W	F	Civ. Dep.	Tokyo U.S. No. 543	0	3	27 Jul	B	6 Aug	None	Mild	Recovery

No.	Complement Fixation — Date Specimen Drawn and Result						Neutralization Index — Date Specimen Drawn and Result						Remarks
	1	2	3	4	5	6	1	2	3	4	5	6	
1	5 Aug 0	12 Aug 1:4(1)	19 Aug 1:32(3)				5 Aug 630	12 Aug 200	19 Aug 10,000				
2	10 Aug 0						10 Aug 320						Virus isolated
3	30 Aug 1:16(4)	9 Sep 1:128(3)	13 Sep 1:128(4)	23 Dec 1:64(2)			30 Aug 1,000	9 Sep 20,000	13 Sep 100,000	23 Sep 20,000			
4	11 Aug 1:4(1)	23 Aug 1:128(4)	31 Aug 1:256(3)	8 Sep 1:256(4)	21 Dec 1:32(3)		11 Aug 1,600	23 Aug 1,600	31 Aug 63,000	8 Sep 32,000	21 Dec 50,000		
5	17 Aug 1:16(4)	30 Aug 1:32(2)					17 Aug 3,200	30 Aug 6,300					
6	2 Sep 0	13 Sep 1:8(4)	23 Sep 1:32(3)				2 Sep 630	13 Sep 20,000	23 Sep 2,000				Some residual
7	18 Aug 1:8(2)	30 Aug 1:32(3)					18 Aug 10,000	30 Aug 200,000					
8	28 Aug 1:4(2)	7 Sep 1:256(4)	29 Dec 1:64(3)				28 Aug 10,000	7 Sep 200,000	29 Dec 400,000				
9	24 Aug 1:8(3)	13 Sep 1:16(4)					24 Aug 1,300	13 Sep 63,000					Arrived Japan 8 July 1948
10	20 Aug 0	3 Sep 1:16(4)	22 Sep 1:16(3)	26 Oct 1:16(4)			20 Aug 200	3 Sep 2,000	22 Sep 20,000	26 Oct --			Protracted recovery
11	9 Aug 0	23 Aug 1:16(3)	31 Aug 1:16(3)	7 Sep 1:16(4)			9 Aug 500	23 Aug 16,000	31 Aug 6,300	7 Sep 32,000			Protracted recovery
12	21 Aug 0	1 Sep 1:16(2)	10 Sep 1:32(3)	13 Sep 1:32(3)			21 Aug 20	1 Sep 13,000	10 Sep 20,000	13 Sep 32,000			Slow recovery
13	23 Aug 0	3 Sep 1:16(3)	22 Sep 1:16(4)	26 Oct 1:8(2)			23 Aug 10,000	3 Sep 32,000	22 Sep 20,000	26 Oct --			Arrived Japan 8 June 1948
14	30 Aug 0	7 Sep 1:8(3)	10 Sep 1:32(3)	11 Sep 1:16(4)	13 Sep 1:16(4)		30 Aug 6,300	7 Sep QNS	10 Sep 20,000	11 Sep 5,000	13 Sep 6,300		
15	23 Sep 1:16(4)						23 Sep 63,000						Hospitalized Bahrien
16	11 Aug 1:64(2)	23 Aug 1:32(4)	31 Aug 1:32(2)	7 Sep 1:16(4)			11 Aug 3,200	23 Aug 3,200	31 Aug 100,000	7 Sep 32,000			Slow recovery
17	27 Aug 1:16(4)	2 Sep 1:64(4)	13 Sep 1:64(3)	23 Sep 1:64(3)	13 Jan 1:32(4)		27 Aug 320	2 Sep 1,000	13 Sep 10,000	23 Sep 2,000	13 Jan 10,000		
18	9 Aug 0	19 Aug 1:32(3)	10 Sep 1:64(4)	23 Dec 1:8(3)			9 Aug 20,000	19 Aug 63,000	10 Sep 100,000	23 Dec 6,300			
19	2 Sep 0	13 Sep 1:8(4)	30 Sep 1:64(3)	29 Dec 1:8(3)			2 Sep 20,000	13 Sep 13,000	30 Sep 200,000	29 Dec 100,000			
20	23 Aug 1:64(3)	29 Aug 1:32(3)	31 Aug 1:32(2)	21 Dec 1:8(3)			23 Aug 100,000	29 Aug 63,000	31 Aug 63,000	21 Dec 40,000			
21	30 Jul 0	6 Aug 1:32(2)					30 Jul 1,000	6 Aug 1,000					
22	18 Aug 1:8(4)	27 Aug 1:16(4)	14 Sep 1:32(4)				18 Aug 20,000	27 Aug 2,400	14 Sep 100,000				
23	11 Aug 0	12 Aug a/c					11 Aug 630	12 Aug 2,000					Virus isolated
24	20 Aug 1:4(1)	27 Aug 1:4(4)	2 Sep 1:32(2)	13 Sep 1:32(2)	23 Sep 1:64(4)	13 Jan 1:32(4)	20 Aug QNS	27 Aug 10,000	2 Sep 10,000	13 Sep 200,000	23 Sep 320,000	13 Jan 10,000	Some residual
25	13 Aug 1:16(4)	23 Aug 1:128(2)	31 Aug 1:64(4)	21 Dec 1:16(3)			13 Aug 320	23 Aug 10,000	31 Aug 100,000	21 Dec 3,200			Arrived Japan 11 July 48
26	20 Aug 1:64(2)	27 Aug 1:256(2)	9 Sep 1:32(3)				20 Aug 6,300	27 Aug 13,000	9 Sep 13,000				
27	16 Aug 1:8(4)	26 Aug 1:32(3)	31 Aug 1:128(4)	13 Sep 1:64(3)	21 Dec 1:16(3)		16 Aug QNS	26 Aug 100,000	31 Aug 130,000	13 Sep 80,000	21 Dec 5,000		
28	20 Aug 0	27 Aug 0	2 Sep 0	14 Sep 1:8(3)			20 Aug 1,000	27 Aug 3,200	2 Sep 3,200	14 Sep 10,000			
29	6 Aug 0	9 Aug 0					6 Aug 100	9 Aug 320					Virus isolated
30	17 Aug 0	30 Aug 1:8(4)	13 Sep 1:16(4)	26 Oct 1:16(3)	20 Dec 0		17 Aug QNS	30 Aug 3,200	13 Sep 10,000	26 Oct --	20 Dec 3,200		Arrived Japan July 1948
31	10 Sep 1:8(4)	22 Sep 1:16(3)	23 Dec 0				10 Sep 10,000	22 Sep 10,000	23 Dec 5,000				

IX. Certain Aspects of Japanese B Encephalitis in Occupation Personnel in Japan and Okinawa 1948

During the summer of 1948 the first recognized American cases of Japanese B encephalitis were observed in Japan. Cases of this disease also occurred in Okinawa during 1948. Earlier reports have described the disease in Americans in Okinawa in 1945 (23) and in Korea in 1946 (36).

The primary purpose or this presentation is to attempt to evaluate certain epidemiological aspects of the small outbreak of encephalitis in Americans in 1948, with particular reference to the effect or non-effect of vaccination as practiced in the field in 1948 in Japan.

1. Case Incidence - The first American case on Okinawa had an onset date or 25 July at the time native cases were most abundant. The first American case in Japan (Tokyo) had an onset date of 4 August 1948 (See Section VIII for Japanese data) while the last reported case (Yokosuka) had an onset date of 28 August 1948.

Twenty-nine serologically confirmed cases occurred in Americans in Japan or were contracted in Japan. Two cases with serological proof occurred in Okinawa. A summation of the presently available data pertaining to the history or vaccination, the date of onset, the clinical course, the outcome, and the serologic results is given in Table 19. Table 20 summarizes incidence data.

Table 20. American Cases of Japanese B Encephalitis 1948

	Army	Navy	DAC	Air Force	Adult Dep.	Other Dep.	Total
Tokyo	7 (2)		1	0	2	2 (1)	12
Tokyo Area	0	0	0	4	0	0	4
Yokohama	5	0	0	0	0	0	5
Yokosuka	0	5[x]	0	0	1	0	6
Other[xx]	2	0	0	0	0	0	2
Total Japan	15	5	1	4	3	2	29
Okinawa	2 (1)	0	0	0	0	0	2
Total Far East	17	5	1	4	3	2	31

[x] Includes one case apparently contracted in Japan but becoming symptomatic in Arabia. Does not include one fatal case apparently contracted in Japan but dying in Ceylon.

[xx] One case each Kobe and Nagoya

Parenthetical figures indicate fatalities.

Because of the small number of cases occurring in any single group case rates are of little comparative value. The rate among one segment of the military population of Tokyo was 53/100,000, the rate among another segment (military) adjacent to Tokyo was 33, the rate among adult dependents in Tokyo was 57.5, and the rate among other dependents in Tokyo was 53. The total Tokyo rate was 44. The total for Tokyo and the adjacent areas was 38. The case fatality rate for the Tokyo area was 17.5% (3/17). In addition to the variations inherent in the small numbers involved, care must be taken in attempting to compare these rates, either among themselves, or with Japanese rates because of a variety of factors, including age differences, sex differences, occupation, exposure hazard, etc.

2. Clinical Aspects - It is not the purpose of this paper to describe in detail the clinical aspects of these American cases. Adequate descriptions have already been cited and similar discussion may be found in TB MED 181. In contrast to the Japanese cases, the course of illness seemed more stormy, hyper-irritability and motor activity were more in evidence in the acute phases of the illness, while a clouded sensorium was present in practically all cases for varying periods at the onset. With one exception (no examination was made) all cases showed a pleocytosis, the total values ranging from 32-925. In the three fatal Tokyo cases the course was one of rapid progression with death occurring within 3 to 5 days after the onset of symptoms. The fatal Okinawa case, a colored male, had an apparent mild form of the disease, appeared to partially recover for a period of one week, and then made a sudden

exitus. In all instances, autopsy revealed an extensive demyelinizing encephalomyelitis with multiple foci of involvement in the brain and spinal cord.

3. Incubation Period - Two cases developing in unvaccinated Navy personnel perhaps give some indication of the incubation period. Two firemen of a Navy ship spent one night ashore in Yokohama in a Japanese operated establishment. The ship was in Yokohama for only two days and sailed on 1 August 1948. Mosquitoes were noted aboard ship while in port and it is stated that three days were required to rid the ship of mosquitoes after sailing. On 11 August one of the men, Kx., reported to the sick bay complaining of a headache of four hours duration. About 30 hours later he was transferred to the British Hospital in Ceylon and died approximately 24 hours after the transfer. He had appeared very drowsy and developed hyperthemia. No autopsy was done.

On 21 August the other fireman, G., reported to the sick bay with a complaint of headache. Temperature of 104° F. was noted and he was "mentally disoriented". He was transferred to a hospital at Bahrein Gulf where a spinal puncture showed a slight pleocytosis and increase in protein. He ran a course similar to the cases observed in Japan. Following recovery he returned to Japan and approximately one month after onset of illness his complement-fixation titer was 1:16 and the neutralization index was 63,000.

4. Severity of Illness - All cases have been categorized as mild, moderate, and severe. With the exception of the early fatal cases no difference is apparent between the vaccinated and non-vaccinated. The post-encephalitic status was assessed by Dr. Charles Aring and his report submitted elsewhere. At this time, only one case (D'Entremont) is known definitely to have had apparent residual symptoms.

5. Serology - Of the sera examined from American personnel, 2 of 13 (15%) from individual cases were positive in complement-fixation tests between the first and fifth days after onset (both on third day). From the 6th through the 10th day, 6 of 10 specimens were positive, while 10 of 11 sera tested between the 11th and 15th day of disease were positive. Only 1 of 24 cases in which specimens were available for testing remained negative after the 10th day. Serum from this case was negative on the 17th day and positive on the 29th day of illness. Many of the other specimens could have become positive at an earlier date, and more could have remained negative at a later date than suggested above. However, since the average interval of time between specimens was 10 days, this question cannot be resolved on the basis of present information. Within the limits imposed by the sampling period there is no apparent difference between the vaccinated and unvaccinated so far as time of appearance of complement-fixing antibodies nor as to the titers reached.

With one exception (in which an equivocal level was obtained) all first samples tested contained neutralizing antibodies. This includes the fatal cases. Such a finding has been previously reported by Sabin in Americans (36) but is contrary to previous Japanese reports. Again, within the limits of the sampling interval no difference is apparent between the vaccinated and the unvaccinated so far as time of appearance of neutralizing antibodies. The maximal levels obtained appear to be slightly higher in the vaccinated individuals. (Note: The levels obtaining in instances of known infection are far higher on the average than are obtained following vaccination alone in adults).

Of 10 patients available for re-testing in December 1948, four showed a decline in complement-fixing and neutralizing antibodies, two showed a disappearance of complement-fixing antibodies and a decline of neutralizing antibodies while four showed a decline of complement-fixing antibodies and either an increase or no significant change in the neutralizing antibody levels.

6. Specificity of Serologic Reactions in Americans - Material presented in the preceding sections indicates that, even using potent vaccine, complement fixing antibodies of the titers presented by most of these cases are not encountered following vaccination in American adults. Serum samples from approximately 30 cases of poliomyelitis (as evidenced by clinical findings of paralysis, clear sensorium, etc., with residual paralyses) even when previously vaccinated, seldom show complement fixing antibodies, and in the few instances where this has occurred titers have never exceeded 1:4 and no evidence of a rise in succeeding samples has obtained. Samples from a miscellany of other diseases have been negative, except for one case of bacterial endocarditis, who gave a history of repeated vaccination for Japanese B encephalitis and a long period of exposure when inapparent infection could have occurred. This individual showed a single titer or a very weak reaction at 1:8.

Although considered much less indicative of an actual infection, all patients in this group called "encephalitis" have demonstrated definite rises in neutralizing antibodies, usually to levels seldom, if ever, encountered as a result of vaccination alone. These findings, coupled with a changing and protean neurologic picture with a clouded sensorium, are considered adequate grounds for diagnosis.

Finally, in three of the four fatal cases, virus isolates were obtained, two in first passage mice. These virus isolates behave serologically in a fashion suggesting their identity with the Nakayama strain

White mice showing paralytic manifestations four days after intracerebral inoculation with established isolate of Japanese B encephalitis. Tokyo 1948

of Japanese B encephalitis.

Whether or not sero-negative cases of Japanese B encephalitis occurred can not be definitely answered. Competent clinicians saw most, if not all, of the cases presenting neurologic evidence of central nervous system involvement in Americans in Japan and Okinawa during 1948. The large majority of these cases without serologic evidence of Japanese B encephalitis had clinical pictures or residual paralyses definitely suggestive of some other type of neurological infection.

7. Vaccination in Americans - During 1945 and 1946 vaccination against Japanese B encephalitis in the Pacific area was done with a mouse brain commercial vaccine. This varied widely in potency (See Section III). During 1947 most of the vaccine administered in Japan was chick-embryo type prepared by AMDR&GS. The prescribed course during 1947 was 1.0 ml. (subcutaneous), 0.1 ml. (intracutaneous), and 1.0 ml. (subcutaneous) on the first, eighth, and 28th days. Many individuals received an additional dose. The acceptable ID_{50} for the vaccine during 1947 was 0.01 ml. and most of the vaccine on delivery approximated this level.

During 1948 all available vaccine was chick-embryo type manufactured by AMDR&GS. The acceptable ID_{50} was 0.02 ml. and most of the vaccine approximated this figure on delivery. Pre- and post-season assays of some of the vaccine used are given in Table 4, Section III. The prescribed course during 1948 was 1.0 ml. subcutaneously on the first, eighth and 28th days. The program was begun in Okinawa on 1 May, in Southern Japan on 15 May, and in the remainder of the theatre on 1 June. Recall doses of 1.0 ml. were given to previously vaccinated individuals.

Previous Sections have dealt with the antibody response induced by vaccination in Americans, both initially and as a recall administration. It must be emphasized that these pre-season figures cannot be directly compared with the probable response engendered during the regular vaccination program since there was a definite difference in the potency of the vaccine used. The available vaccine in the pre-season trial had an ID_{50} of 0.008, or roughly three times the potency of that available for general use. Further, the vaccine is known to deteriorate rapidly after hydration and it is probable that the pre-season trials were conducted under far better conditions than prevailed in many of the stations administering mass vaccinations.

8. Relationship of Vaccination to Disease Incidence - Prevailing conditions were not such as to permit a mass trial of the vaccine by administration to a part of the Americans and withholding the vaccine from a comparable group. However, there were various irregularities in the program, and as expected, a certain percentage of Americans were not completely vaccinated. Two groups, of American military personnel in the Tokyo area, in which other variations have been reduced to a minimum, were analyzed to ascertain the relationship of vaccination to disease incidence. In one group of 14,957 individuals, 3.5% of the vaccination records were examined. In the second group of 12,107, 1.3% of the vaccination records were examined. These were arbitrarily divided into four categories: A. Those with complete vaccination histories; B. Those lacking only one dose (unless that dose was the 1948 recall dose); C. Those receiving less vaccine than Category B.; D. Those receiving no vaccine. Then a similar division was made for known cases of encephalitis occurring in these two groups. The results are presented as Table 21. (In the second part of Table 21 Categories A. and B. have been treated as a unit and termed "adequately vaccinated", while Categories C. and D. have been similarly treated and termed "inadequately vaccinated".

These figures deserve some additional qualification. In each of the two groups the percentages used to divide into the four vaccination categories were those obtained on initial surveys immediately post-season. Other spot checks have indicated that categories C. and D. may well be smaller than these surveys would indicate. Further, the interviewing officers have been repeatedly advised that due to lack of immunization registers, or for other reasons, vaccine was actually administered to individuals without recording the procedure on the individual immunization record. There is little evidence to indicate that the converse, i.e. falsification of registers, occurred on anywhere near the same scale. Consequently, Categories C. and D. probably contain fewer individuals than indicated above. Repeated checks and careful questioning, including careful examination of all possible available records by several examiners, were used to categorize the actual encephalitis cases by vaccination histories, and where there existed any reasonable question, credit was given for administration of the vaccine.

Five of the patients (Dawson, D'Entremont, Gorman, Myers, and Wright) received vaccine, one or more doses, within ten days prior to onset of their illness. In several of these five the time interval was much shorter. Such vaccine administration was not considered in the division into categories. Similarly, vaccine administered to any other person in the Groups listed above after 15 July 1948 (20 days prior to 4 August 1948, the date of onset of the first American case) was not considered in the division into categories.

Table 21. Relationship of Vaccination to Disease Incidence in Two Segments of Military Population of Tokyo Area 1948

	A	B	C	D	Total
Group I	10,769 (72%)	2,243 (15%)	1,495 (10%)	448 (3%)	14,957 (100%)
Cases	3	1	1	3	8
Rate[x]	27.9	45.6	66.8	670	53
Group II	2,403 (20%)	5,407 (45%)	2,102 (17.5%)	2,102 (17.5%	12,017 (100%)
Case	1	0	2	1	4
Rate[x]	41.6	0	95.0	95.0	33

	"Adequately Vaccinated"	"Inadequately Vaccinated"
	A & B	C & D
Group I	13,012 (87%)	1,944 (13%)
Cases	4	4
Rate	30.7	154.0
Group II	7,811 (65%)	4,205 (35%)
Cases	1	3
Rate	12.8	71.3

[x] Rate per 100,000
See text for explanation of categories A., B., C., D.

I. Expected cases in vaccinated at rates of total - 8/14,957 X 13,012 = 7.4
Expected cases in vaccinated at rates of unvaccinated - 4/1,944 X 13,012 = 26.8
Actual cases in vaccinated = 4.0

II. Expected cases in vaccinated at rates of total - 4/12,017 X 7,811 = 2.6
Expected cases in vaccinated at rates of unvaccinated - 3/4,205 X 7,811 = 5.6
Actual cases in vaccinated = 1.0

It is therefore believed that the figures on expected case incidence in the vaccinated categories A. and B. have been derived in a fashion intended to subject the vaccine to as critical an evaluation as is possible. That there are inherent fallacies, due to the small number of cases, is fully recognized. (Actually one large organization, contributing the bulk of the personnel in Group I. had no cases in the completely vaccinated Category A.)

The remaining cases of Japanese B encephalitis occurring in Americans were in variegated groups, almost completely lacking in homogeneity, and the number of cases per group is unsatisfactory for further analysis. Using the categories described above the entire group may be broken down as follows:

Category A.	(Complete Vaccination)	14/31 (45%)	1 death (Okinawa)
Category B.	(Complete except for one dose)	3/31 (9.7%)	
Category C.	(Less vaccine than B.)	6/31 (19.3%)	1 death (Tokyo)
Category D.	(No vaccine)	8/31 (26%)	2 deaths (Tokyo)

Of the 14 individuals listed in Category A. seven had received a previous immunization in 1947 with a recall dose in 1948, while the other seven were initially vaccinated in 1948.

9. Summary - Data pertaining to the general aspects of Japanese B encephalitis occurring in Occupation personnel in Japan and Okinawa in 1948 have been presented.

An attempt has been made to evaluate the possible effectiveness or non-effectiveness of the previous mass-immunization with Army Medical Department Research and Graduate School lyophilized chick-embryo Japanese B encephalitis vaccine. Clinical cases of encephalitis were derived almost equally from the group considered vaccinated and the group considered not adequately vaccinated, while in total numbers the vaccinated group far exceeded the not adequately vaccinated.

To obtain cases occurring in relatively homogenous groups under similar conditions of exposure evaluation of case incidence has been made in two relatively homogenous military groups in the Tokyo area.

In one segment of the military population 13,012 individuals considered to be adequately vaccinated had 4 clinical cases of encephalitis while a similar inadequately vaccinated group of 1,944 also had 4 cases. In a second segment of military population 7,811 individuals considered to be adequately vaccinated had 1 clinical case of encephalitis while a similar inadequately vaccinated group of 4,205 individuals had 3 cases.

One (Okinawa) of the 4 fatal cases had received a full series of vaccine in 1948. One (Tokyo) had received a full vaccination series in 1947 but no recall dose in 1948. The other two fatal cases had received no vaccine.

In the small number of cases available for study no difference between the vaccinated and non-vaccinated could be observed in the clinical course or in the development of specific antibodies.

Conclusion - While on the basis of the small numbers of cases presented it would appear that some protection was engendered by the vaccine, it is not believed that any more definitive statement is warranted.

X. Japanese Equine Encephalomyelitis

Practically no reports have appeared in other than Japanese literature concerning the equine encephalomyelitis of Japan. Because of the economic importance of this disease, and because of the probable similarity to Japanese B encephalitis in humans, a comprehensive summation has recently been prepared by Burns and Matumoto (73). Cases of this summer disease of equines have apparently been known since 1894. Since that time at intervals of several years sufficient numbers of cases have occurred to warrant an epizootic description. During the summer and early fall of 1935 cases occurred on all the main islands of Japan including Hokkaido. At least 1,180 cases were reported and many more were considered to have occurred.

1. Clinical Aspects - The onset of this malady may occur with explosive abruptness or it may follow premonitory symptoms of malaise, reduced appetite, etc. The temperature may rise very rapidly to 41° C. and is usually followed by symptoms of meningo-encephalomyelitis. Concomitantly with appearance of symptoms referable to the central nervous system, the temperature frequently recedes, even to the normal level.

Cases with a predominant involvement of the central nervous system present a characteristic excitation which later may pass to depression and apathy, and even to pronounced spastic or flaccid types of paralysis. In other cases in which the spinal cord is primarily involved, flaccid paralysis or weakness may be observed without any indication of a disturbed sensorium. In the cerebral type of case the animal may be completely disoriented, kicking, rearing, or receiving extensive bodily injury without any indication of pain. As the disease progresses weakness or paralysis appears more pronounced and the animals sink quietly into depression and expire. Difficult deglutition, fibrillar spasm of the muscles of the limbs, sightlessness and paresis of tongue and lips are common. Milder forms may be observed.

2. Epizootiology - a. 1947 - Following the first reported encephalitides type infection among equines on 10 May 1947 from Kochi Prefecture (Shikoku) case reports were submitted from other islands

of Japan (except Hokkaido). Cases were reported during the months of July, August, September, and October (6).

Reported cases totaled 1,209 with a morbidity rate of 1.84 per cent if the actual population of equines in areas where cases occurred is used as a base. Males, females and gelding appeared to be involved equally. A large number of cases occurred among younger animals. The peak of distribution lies at 3 years of age, with approximately 80% of all cases being found in the 2 to 4 year age group.

For all Japan 611 equine deaths were reported. Approximately 1 animal per 100 equine population of areas where cases occurred succumbed to an encephalitides type infection. The case fatality rate was 50.5 per cent.

b. 1948 - As in 1947 an encephalitides type infection among equines appeared in epizootic proportions. Initial onset was reported as being on the 30th of May; however it was not until 16 July that these opinions could be confirmed (Isolate V-2900). The latter date was 60 days prior to the expected seasonal appearance of Japanese equine encephalomyelitis.

This malady was soon reported throughout Kyushu, Shikoku, and Honshu, the only exception being the prefectures of Aichi, Osaka, Hyogo, Mie and Nara. It is interesting to note that five prefectures reported no cases during the 1947 epizootic. It is not known whether this is due to failure to recognize cases or to a specific peculiarity of animal non-infectivity. (Reports of human cases leaves no doubt that the disease existed in this area in epidemic proportions during 1948.)

From initial onset until the last reported equine case of 8 November 1948, there were 3,697 cases with 1,367 deaths and 100 sacrifices - a fatality rate of 39.6 percent (8).

Heretofore Hokkaido has been considered a non-enzootic area for Japanese equine encephalomyelitis, although a few cases had been reported in 1935. Studies (27) in 1946 had shown serologic evidence of the disease in equines. On 24 August an outbreak of epizootic proportions occurred among equines in Hokkaido. The final case report was forwarded on the 8th of November 1948. A total of 785 cases was reported (8) with 245 deaths and 27 sacrifices, a fatality rate of 34.5 per cent.

The attack rate was highest among young animals with mortality being the greatest among the older age groups. The disease appeared to be disseminated throughout the island areas in direct ratio to the concentration of equine populace. Brain tissues of 2 animals were received but no agent has been recovered.

3. Serological and Etiological Studies - In 1947 two strains (Nos. 2683 and 2869) of neurotropic viruses were isolated in mice from brain tissues of equines which were clinically diagnosed as having an encephalitides type of infection. In each case, isolation was attempted three times and was successful twice. In the last attempts the brains had been stored in a 50% glycerine saline mixture at 4° C for an extended period of time (No. 2683 - 85 days, No. 2869 - 41 days).

Antigens prepared from isolates 2683 and 2869 fixed complement in the presence of Japanese B (Nakayama strain) immune guinea pig serum in the same titre as that of the Japanese B (Nakayama) virus antigen, while they failed completely to react with St. Louis encephalitis and Western equine encephalomyelitis immune sera. Cross-neutralization tests demonstrated that Japanese B (Nakayama) immune rabbit sera neutralized its homologous virus and viruses 2683 and 2869. Some evidence of cross-neutralization with the heterologous virus of St. Louis encephalitis was noted both with the control Nakayama and the isolates. Western and Easterm types of equine encephalomyelitis viruses were not neutralized. Rabbit hyperimmune sera produced against the viruses 2683 and 2869 gave results similar to Japanese B immune sera. St. Louis immune sera had some neutralizing capacity for the Nakayama virus and the two equine isolates but showed a much higher neutralization index with the homologous virus.

Mice vaccinated with Japanese B encephalitis (Nakayama) vaccine were resistant to intracerebral inoculations of virus of 2683 and 2869, as well as to a homologous challenge. Vaccinated mice of this group challenged with the virus of St. Louis encephalitis showed a resistance index of only 25. There was no protection against Eastern and Western equine encephalomyelitis viruses. Similar results were obtained in mice vaccinated with formalin inactivated viruses 2683 and 2869. Thus, complement-fixation, neutralization and resistance tests would indicate that the two equine isolates were similar to, if not immunologically identical with, the virus of Japanese B encephalitis (Nakayama strain) in human beings. During the epizootic of 1947 clinical cases of Japanese B encephalitis were observed among humans in the same geographical areas and three strains (Nos. 2285, 2311, and 2388) were isolated from fatal human cases. Serologically these strains are very similar, if not identical to the equine isolates and to the Nakayama strain. Immunization studies are still in progress.

Complement fixation tests conducted periodically during the summer of 1947 would indicate that complement fixing antibodies for the virus of Japanese B encephalitis in equines occur as a result of recent infection with this virus. (Earlier work had indicated that both complement-fixing antibodies and neutralizing antibodies develop in equines without obvious evidence of disease when serial samples were obtained in enzootic areas. It had been further shown that animals with negative complement-fixation but positive neutralizing antibodies at the beginning of the summer season could develop complement-fixing antibodies during the summer months.)

In most instances sera of equines suffering from an encephalitides type infection gave specific complement fixation against the virus antigen of Japanese B encephalitis. There was a high incidence of complement fixing antibodies for Japanese B virus among normal horses in epizootic areas, suggesting an extensive inapparent spread of the virus during 1947. In a control group of normal horses in Hokkaido, where no cases were reported during 1947, only one of 39 equines showed complement fixing antibodies for the Japanese B virus. These data support the opinion that the virus of Japanese B encephalitis is the etiological agent of this epizootic.

Since Japanese B encephalitis did not occur in epidemic form among humans in 1947, it is further evident that the disease may occur in epizootic form in equines, and that numerous inapparent infections may occur in equines, without a concomitant occurrence of the disease in epidemic form in humans.

4. Equine Vaccination Program - A field trial to test the value of Japanese equine encephalomyelitis vaccine was undertaken to determine the serological response of horses following vaccination with this type of biologic.

Twenty-five horses repeatedly tested, and found to be devoid of neutralizing antibodies for the virus of Japanese B encephalitis, were vaccinated subcutaneously with a formalinized vaccine. This biologic was prepared in this laboratory from mouse brain tissue infected with the virus (isolate No. 2683 and 2869) of Japanese equine encephalomyelitis. Ninety-two percent of the vaccinated equines developed neutralizing antibodies to the homologous virus and to the Nakayama strain. A complete summation is shown in Table 22.

Complement fixing antibodies for the normal mouse brain component were produced as well as for those of the virus. Following vaccination only 2 of the 25 horses demonstrated a significant and specific increase in complement fixing titer to the virus of Japanese B encephalitis and Japanese equine encephalomyelitis.

Table 22. Equine Vaccine Program - Isolates 2683 and 2869

Schedule of Vaccination			ID_{50} of vaccine when challenged with:		
	Tokyo	Chiba	Nakayama	2683	2869
1st Vacc.	25 Feb.	24 Feb.	(0.0030 (8.0)[x]		0.0021 (7.3)
			(0.0024 (8.0)		0.0012 (7.3)
2nd Vacc.	4 Mar.	5 Mar.)			
		)	0.0012 (8.1)	0.0035 (8.3)	0.0012 (8.0)
3rd Vacc.	15 Mar.	16 Mar.)			
Bleeding	27 May	28 May			

[x] Figures in parenthesis represent LD_{50} of challenge virus used in potency tests

Neutralization Titers Elicited by Administration of Mouse Brain Vaccine to Equines
(Positive represents an index of 50 or more, equivocal is 10-49, negative is less than 10)

Dosage of Vaccine:
A group: 2 ml / 2 ml / 4 ml
B group: 4 ml / 4 ml / 8 ml

	Tokyo								Chiba								Tokyo & Chiba							
	Nakayama				No. 2683				Nakayama				No. 2683				Nakayama				No. 2683			
	/	/	-	Tot	/	/	-	Tot	/	/	-	Tot	/	/	-	Tot	/	/	-	Tot	/	/	-	Tot
Group A	5	3	0	8	8	0	0	8	3	0	3	6	4	1	1	6	8	3	3	14	12	1	1	14
Group B	6	1	0	7	7	0	0	7	2	1	1	4	3	0	1	4	8	2	1	11	10	0	1	11
Total	11	4	0	15	15	0	0	15	5	1	4	10	7	1	2	10	16	5	4	25	22	1	2	25
	15				15				6				8				21				23			

Summary - In very brief fashion some of the salient features of the equine encephalomyelitis of Japan have been presented. Comprehensive studies have been submitted for publication elsewhere. It is considered that the causative agent of this disease of equines and the virus of Japanese B encephalitis of humans are immunologically and serologically similar if not identical. (Note: Many blood samples and several isolates obtained during the summer of 1948 have not been studied up to this time because of the press of other work. Preliminary tests would suggest that additional support for the hypotheses advanced above will be obtained).

XI. Serological Pattern in Japanese Following the 1948 Epidemic of Japanese B Encephalitis

As has been noted in previous sections inapparent infections, based primarily on the finding of neutralizing antibodies in a large portion of certain populations in the Far East, are considered to be extremely common during both epidemic and non-epidemic years. However, no large scale studies were available, in which both complement-fixing antibodies and neutralizing antibodies had been determined in Japan after an outbreak of the magnitude of that seen in 1948. Consequently it was considered desirable to ascertain the serologic pattern existing in both Japanese and Americans in the Tokyo area in late 1948.

Materials and Methods - As a cooperative project with the Japanese National Institute of Health blood samples were obtained from cross-sections of the indigenous population of Greater Tokyo. After processing and freezing the samples, neutralization and complement fixation tests were carried out according to methods previously outlined. This study is not yet completed and this material is presented as a progress report.

Results - Table 23 summarizes the presently available information. (The location of the areas sampled may be found by reference to Figure 7). As a base line the figures obtained by Deuel and Bawell in late 1946 have been included. The individuals tested in 1948 are not the same as those tested in 1946.

Discussion - During 1946 no cases of encephalitis were known to have occurred in Tokyo. Likewise, no significant number of cases are known to have occurred in 1947. However, concurrently with the epizootic previously described studies made on humans in rural areas adjacent to Tokyo (Gifu and Chiba) showed detectable complement fixing antibodies in 27 of 56 individuals tested. Consequently, it is possible that the rural area of Jindai-mura may represent a composite of two years infection since 1946.

In so far as the groups tested are representative samples of the populations of the areas from which they were drawn, it would appear that there is a definite difference in the numbers of individuals possessing complement-fixing antibodies after exposure during the summer of 1948. It would further appear that the difference between the numbers of individuals in urban Tokyo possessing neutralizing antibodies in 1946 and in 1948 can be represented by the number of individuals possessing complement-fixing antibodies in the post-season survey of 1948.

If complement-fixing antibodies actually represent a true measure of the extent of inapparent infection in the months immediately preceding the time the test samples are drawn, there were wide variations in the degree of exposure in various areas of Tokyo. Additional studies to inquire into this apparent difference and to evaluate the assumption on which this theory is based are in progress.

XII. Japanese B Encephalitis Studies in Progress

Because of the time consuming nature of many of the studies, much of the 1948 material has not been completed. Brief reference to these have been made in some of the earlier sections and additional projects are noted below.

1. Serological pattern in Occupation Personnel in Tokyo Area - Blood samples were obtained post-season on various units in the Tokyo area. Preliminary complement-fixation tests would indicate that from seven to fifteen percent of these troops will show complement-fixing antibodies.

2. Okinawa - Extensive pre- and post-season collections of blood samples from American and indigenous personnel were made during 1948.

3. Guam - Between 11-26 February members of this unit participated in an investigation of an outbreak of Japanese B encephalitis occurring on Guam. This was the first known outbreak to have occurred on Guam. Participating in this investigation was a group derived from three medical units in Japan, the Hooper Foundation, the Naval Epidemiological Unit at Pearl Harbor, the MARBO Army Surgeon's Staff, the Navy Medical Center in Guam, and the Naval Civil Government of Guam.

Table 23.

Incidence of Neutralizing and Complement-Fixing Antibodies Against Virus of Japanese B Encephalitis in Tokyo

Japanese Nationals September 1948

Neutralization Index

Age	Chuo-ku Pos. 1946[X]		Daito-ku Equiv. 1948		Daito-ku Pos. 1948		Suginami-ku Equiv. 1948		Suginami-ku Pos. 1948		Ohta-ku Equiv. 1948		Ohta-ku Pos. 1948		Kita-tama Pos. 1946[X]		Kita-tama Pos. 1948	
0-4	2/19	11%	0/8		1/8	13%	1/18	6%	2/18	11%	0/12		2/12	17%	0/19		5/10	50%
5-9	7/21	33%	1/20	5%	2/20	20%	2/14	14%	4/14	29%	0/11		6/11	55%	3/16	11%	8/14	57%
10-14	9/21	42%	1/17	6%	9/17	53%	0/17		3/17	18%	0/20		14/20	70%	10/16	62%	13/19	68%
15-19	12/21	57%	1/15	7%	7/15	47%	0/20		11/20	55%	2/25	8%	17/25	68%	15/22	68%	13/20	65%
20-39	12/13	92%	0/23		20/23	87%	0/18		16/18	89%	0/29		24/29	83%	12/13	92%	18/18	95%
40-59	16/16	100%	0/11		11/11	100%	2/19	11%	16/19	84%	0/20		19/20	95%	17/17	100%	19/21	91%
60 +	20/20	100%	0/7		6/7	86%	0/20		19/20	95%	0/6		5/6	83%	17/17	100%	19/19	100%

Complement Fixing Antibodies

Positive Tests

0-4	0/8		1/18	6%	3/16	19%	4/13	31%
5-9	1/21	5%	1/14	7%	3/13	23%	3/20	15%
10-14	1/17	6%	2/18	11%	2/20	10%	2/20	10%
15-19	0/15		0/20		1/25	4%	3/20	15%
20-39	0/24		1/19	5%	4/29	14%	3/21	14%
40-59	0/11		0/10		1/20	5%	0/21	
60 +	1/7	14%	0/20		1/7	14%	1/19	5%

[X] 1946 data is quoted from Reference 27.
Kita-tama is a rural area near Tokyo. The remainder of the tabulation covers samples drawn in urban Tokyo.

GUAM SUMMARY

Group I: Talofofo Contacts

	Positive		Equivocal		Negative	
N.I.	13/28	(46%)	1/28	(4%)	14/28	(50%)
C.F.	21/38	(55%)	--		17/38	(45%)

Age Distribution

	N.I. Pos.	N.I. Equiv.	N.I. Neg.	C.F. Pos.	C.F. Neg.
<1-4	-	-	-	2/3	1/3
5-9	2/4	0/4	2/4	4/6	2/6
10-14	5/9	0/9	4/9	7/10	3/10
15-19	2/2	0/2	0/2	2/3	1/3
20-29	2/7	0/7	5/7	2/9	7/9
30-39	1/2	1/2	0/2	2/2	0/2
40/	0/1	0/1	1/1	0/1	1/1
?	0/1	0/1	1/1	0/1	1/1

Group II: Talofofo Non-Contacts

		Pos.		Equiv.		Neg.	
N.I.	(2/23/48)	14/21	(67%)	1/21	(5%)	6/21	(28%)
N.I.	(4/14/48)	15/24	(62%)	2/24	(8%)	7/24	(29%)
N.I.	(2 spec.)	15/24	(62%)	2/24	(8%)	7/24	(29%)
C.F.	(2/23/48)	5/13	(38%)			8/13	(61%)
C.F.	(4/14/48)	3/24	(12%)			21/24	(87%)

Age Distribution (Results based on 2 specimens)

	N.I. Pos.	N.I. Equiv.	N.I. Neg.	C.F. Positive 1st	C.F. Positive 2nd	C.F. Negative 1st	C.F. Negative 2nd
<1-4	0/2	0/2	2/2	-	0/2	-	2/2
5-9	3/4	1/4	0/4	-	0/4	-	4/4
10-14	2/3	0/3	1/3	0/1	0/3	1/1	3/3
15/19	4/6	0/6	2/6	2/4	1/6	2/4	4/6
20-29	4/4	0/4	0/4	2/3	1/4	1/3	3/4
30-39	1/2	0/2	1/2	0/2	0/2	2/2	2/2
40/	1/3	1/3	1/3	1/3	1/3	2/3	2/3

Group III: Merizo Native Contacts.

	Positive		Equivocal	Negative	
N.I.	7/9	(78%)	0/9	2/9	(22%)
C.F.	5/9	(56%)	-	4/9	(44%)

Age Distribution

	N.I. Pos.	N.I. Equiv.	N.I. Neg.	C.F. Pos.	C.F. Neg.
1-4	-	-	-	-	-
5-9	3/3	0/3	0/3	2/3	1/3
10-14	-	-	-	-	-
15/19	1/1	0/1	0/1	1/1	0/1
20-29	1/2	0/2	1/2	1/2	1/2
30-39	-	-	-	-	-
40/	2/3	0/3	1/3	1/3	2/3

Group IV: Merizo Natives - Non-Contacts.

		Pos.		Equiv.		Neg.	
N.I.	(2/20/48)	10/22	(45%)	1/22	(4%)	11/22	(50%)
N.I.	(4/19/48)	15/18	(83%)	3/18	(17%)	0/18	(0%)
N.I.	(2 spec.)	17/22	(77%)	3/22	(14%)	2/22	(9%)
C.F.	(2/20/48)	11/22	(50%)			11/22	(50%)
C.F.	(4/19/48)	2/18	(11%)			16/18	(89%)

Age Distribution (Results based on 2 specimens)

	N.I. Pos.	N.I. Equiv.	N.I. Neg.	C.F. Positive 1st	C.F. Positive 2nd	C.F. Negative 1st	C.F. Negative 2nd
<1-4	3/4	0/4	1/4	1/4	0/3	3/4	3/3
5-9	2/4	0/4	2/4	1/4	0/4	3/4	4/4
10-14	5/5	0/5	0/5	4/5	1/3	1/5	2/3
15-19	3/5	1/5	1/5	2/5	1/4	3/5	3/4
20-29	2/2	0/2	0/2	1/2	0/2	1/2	2/2
30-39	1/1	0/1	0/1	0/1	0/1	1/1	1/1
40/	1/1	0/1	0/1	1/1	0/1	0/1	1/1

Group V: Inarajan Natives

		Pos.		Equiv.		Neg.	
N.I.	(2/20/48)	10/13	(77%)	2/13	(15%)	1/13	(8%)
N.I.	(4/19/48)	7/9	(78%)	2/9	(22%)	0/9	(0%)
N.I.	(2 spec.)	10/13	(77%)	3/13	(23%)	0/13	(0%)
C.F.	(2/20/48)	2/12	(17%)	---		10/12	(83%)
C.F.	(4/19/48)	1/8	(12%)	---		7/8	(87%)

Age Distribution (N.I. based on results of 2 sera where available)

	N.I. Pos.	N.I. Equiv.	N.I. Neg.	C.F. Pos. 1st.	C.F. Pos. 2nd.	C.F. Neg. 1st.	C.F. Neg. 2nd.
<1-4	-	-	-	-	-	-	-
5-9	-	-	-	-	-	-	-
10-14	5/7	2/7	0/7	2/6	1/6	4/6	5/6
15-19	4/4	0/4	0/4	0/4	0/2	4/4	2/2
20-29	0/1	1/1	0/1	0/1	-	1/1	-
30-39	-	-	-	-	-	-	-
40/	-	-	-	-	-	-	-
?	1/1	0/1	0/1	0/1	-	1/1	-

Groups I-V: (Talofofo, Merizo, Inarajan)

	Pos.		Equiv.		Neg.	
(1st Spec.)	54/93	(58%)	5/93	(5%)	34/93	(36%)
(2nd Spec.)	37/51	(73%)	7/51	(14%)	7/51	(14%)
(2 spec.)	62/96	(65%)	9/96	(9%)	25/96	(26%)
(1st Spec.)	44/94	(47%)			50/94	(53%)
(2nd Spec.)	6/50	(12%)			44/50	(88%)

Age Distribution (N.I. based on results of 2 sera)

	N.I. Pos.	N.I. Equiv.	N.I. Neg.	C.F. (1st Spec. only) Pos.	C.F. (1st Spec. only) Neg.
1-4	3/6 (50%)	0/6	3/6 (50%)	3/7 (43%)	4/7 (57%)
5-9	10/15 (67%)	3/15 (20%)	2/15 (13%)	7/13 (54%)	6/13 (46%)
10-14	17/24 (71%)	2/24 (8%)	5/24 (21%)	13/22 (59%)	9/22 (41%)
15-19	14/18 (78%)	1/18 (6%)	3/18 (17%)	7/17 (41%)	10/17 (59%)
20-29	9/16 (56%)	1/16 (6%)	6/16 (37%)	6/17 (35%)	11/16 (65%)
30-39	3/5 (60%)	1/5 (20%)	1/5 (20%)	2/5 (40%)	3/5 (60%)
40/	5/10 (50%)	1/10 (10%)	4/10 (40%)	5/11 (45%)	6/11 (54%)
?	1/2 (50%)	0/2 (0%)	1/2 (50%)	0/2 (0%)	2/2 (100%)

GUAM SUMMARY

Group VI: Yigo Natives (Non-Contacts)

	Pos.		Equiv.		Neg.	
N.I.	4/25	(16%)	4/25	(16%)	17/25	(68%)
C.F.	0/24	(0%)			24/24	(100%)

Age Distribution

	N.I. Pos.		N.I. Equiv.		Neg.	
<1-4	0/5	(0%)	0/5	(0%)	5/5	(100%)
5-9	0/5	(0%)	2/5	(40%)	3/5	(60%)
10-14	1/6	(17%)	1/6	(17%)	4/6	(67%)
15-19	0/4	(0%)	0/4	(0%)	4/4	(100%)
20-29	0/1	(0%)	0/1	(0%)	1/1	(100%)
30-39	1/2	(50%)	1/2	(50%)	0/2	(0%)
40+	2/2	(100%)	0/2	(0%)	0/2	(0%)

Group VII: Marines - Yona Area

		Pos.		Equiv.		Neg.	
N.I.	(2/18/48)	0/26	(0%)	3/26	(11%)	23/26	(88%)
N.I.	(4/14/48)	1/25	(4%)	2/25	(8%)	22/25	(88%)
N.I.	(2 spec.)	1/27	(4%)	2/27	(7%)	24/27	(89%)
C.F.	(2/18/48)	1/27	(4%)			26/27	(96%)
C.F.	(4/13/48)	1/27	(4%)			26/27	(96%)

Group VIII: U. S. Naval Hospital Personnel

	Pos.		Equiv.		Neg.	
N.I.	0/17	(0%)	6/17	(35%)	11/17	(65)
C.F.	0/17	(0%)			17/17	(100%)

Group IX: Guam Memorial Hospital Personnel

	Pos.		Equiv.		Neg.	
N.I.	1/16	(6%)	2/16	(12%)	13/16	(81%)
C.F.	0/16	(0%)			16/16	(100%)

Group X: Guam School of Medical Practitioners

	Pos.		Equiv.		Neg.	
N.I.	3/27	(11%)	5/27	(18%)	19/27	(70%)
C.F.	0/25	(0%)			25/25	(100%)

Group XI: Marama, Harmon Field

	Pos.		Equiv.		Neg.	
N.I.	3/29	(10%)	0/29	(0%)	26/29	(90%)
C.F.	0/29	(0%)			29/29	(100%)

Group XII: Barrigada Animal Series

	Pos.	N.I. Equiv.	Neg.
Doves	0/4	0/4	4/4
Chickens	0/3	1/3	2/3
Carabao	1/2	0/2	1/2
Cow	1/3	1/3	1/3
Horse	2/2	0/2	0/2
Pig	3/3	0/3	0/3
Goat	1/2	0/2	1/2
Dog	0/1	0/1	1/1

Group XIII: Talofofo Animal Series

	Pos.	Equiv.	Neg.
Chickens	0/2	0/2	2/2
Carabao	3/3	0/3	0/3
Cow	3/3	0/3	0/3
Horse	1/1	0/1	0/1
Goat	1/2	0/2	1/2
Dog	1/3	0/3	2/3

Group XIV: Marizo Animal Series

	Pos.	N.I. Equiv.	Neg.
Chickens	0/4	0/4	4/4
Carabao	4/4	0/4	0/4
Cow	1/3	2/3	0/3
Pig	3/3	0/3	0/3
Goat	0/1	1/1	0/1
Dog	2/2	0/2	0/2

Group XV: Guam Rats

Pos.	N.I. Equiv.	Neg.
0/12	1/12	11/12

C.F. 6/6 Negative

Groups XII - XV: Guam Animal Series

	Pos.		N.I. Equiv.		Neg.	
Doves	0/4	(0%)	0/4	(0%)	4/4	(100%)
Chickens	0/9	(0%)	1/9	(11%)	8/9	(89%)
Carabao	8/9	(89%)	0/9	(0%)	1/9	(11%)
Cow	5/9	(56%)	3/9	(33%)	1/9	(11%)
Horse	3/3	(100%)	0/3	(0%)	0/3	(0%)

ISLAND OF GUAM

COMPILED FROM THIRD AIR PHOTO RECONNAISANCE SQUADRON PHOTOGRAPHS AND ROAD SURVEY.

FIG. 9

From five fatal cases (two in Americans) occurring in December and January a neurotropic agent identified as Japanese B encephalitis virus was isolated in this laboratory and at Hooper Foundation. Forty native cases out of a population of approximately 28,000 were identified on epidemiological grounds. The diagnosis was complicated by the concomitant occurrence of an outbreak of mumps but it would appear that the peak incidence occurred during the weeks ending 9 and 16 January.

Cases were confined to the southern half of the island and even these were widely scattered, tending to occur in small focal areas in a village (adjacent houses) and to completely skip some areas.

Investigation included large-scale collections of blood samples from humans and animals, collections of mosquitoes for attempts at virus isolation, and collections of suitable statistical data for final analysis. Approximately one-half of the blood samples were tested in this laboratory and are shown in Table 24. The villages studied are shown in Figure 9.

Because several aspects of this outbreak remain to be clarified, and because a joint report will be made with the other participating agencies no attempts to derive conclusions is made at this time. It would appear that the outbreak was widespread in the southern part of the island, that there is no significant difference between contacts and non-contacts, and that a large proportion of the animals tested showed evidence of experience with the virus.

Plans for 1949

Diagnostic and investigative studies along the lines covered in the preceding sections will continue. These will include large scale vaccine evaluation studies, antigenic studies of various rickettsial and virus isolates, attempts to elucidate the vectors and reservoirs, animal studies to duplicate in so far as possible certain conditions observed in the field under suitable controlled conditions, and such other studies as may be dictated by epidemiological considerations.

"With observations of this kind, limited in time and space, it is well to reflect upon the fact that "if when the tide is falling you take out water with a twopenny pail, you and the moon together can do a great deal.""

Hill, 1948

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7a. The diet consisted of the following ingredients:

Dried cabbage	12.0 lbs.
Rolled oats	15.0 lbs.
Corn meal	20.0 lbs.
Wheat meal	30.0 lbs.
Dried whole milk	12.0 lbs.
Dried whole eggs	6.0 lbs.
Dried yeast	2.0 lbs.
Calcium carbonate (USP)	1.0 lb.
Sodium chloride (ACS)	1.0 lb.
Iron sulfate (USP)	0.25 lb.
Cod Liver oil	1.5 pints

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OFFICERS AND DEPARTMENT OF THE ARMY CIVILIANS

Assigned on Permanent Status

1 January 1948 thru 31 December 1948

Commanding

Tigertt, William D., Lt. Colonel, MC

Administration

Haase, Fredrick J., Major, MSC (Adjutant)
Wilson, Owen D., Captain, MSC (Supply Officer)
Hindley, Frederick W., Captain, MSC (Liaison Officer)
Roberts, Dolores M., CAF-4 (Secretary)
Moore, Gladys, CAF-4 (Stenographer) (Assigned 26 July 1948)

MedicallZoology Section

Hunter, George W. III, Lt. Colonel, MSC, Chief
McMullen, Donald B., P-6
Ritchie, Lawrence S., P-5
Stray, Estelle, P-2
Yamada, Mabel, SP-7

Serology Section

Stein, George J., P-6, Chief
Wahl, Anne, P-2

Chemistry Section

White, Edward A., P-5, Chief
Kaufman, Edwin H., Captain, MC (Departed 10 November 1948)
Stabile, Joseph N., Captain, MSC (Assigned 19 February 1948)
Balikov, Bernard, 1st Lt., MSC

Bacteriology Section

Barksdale, Walter L., P-5, Chief
Sanders, Arvey C., Major, MSC (Assigned 19 June 1948)
Lipscomb, Edward V., Captain, MSC
D'Aniello, Anthony A., 1st Lt., MSC
Hopphan, Ethel, P-5
White, Joan A., P-5
LaFond, Bernice, CAF-3 (Stenographer) (Assigned 6 December 1948)

Pathology Section

Aronson, Roland S., Lt. Colonel, MC, Chief
Wilson, Lincoln E., Captain, MC
Scott, Edwin L., Captain, MC (Assigned 19 February 1948)
Ogilvie, Robert W., Captain, MC (Assigned 23 September 1948)
Acuff, Tea E., 1st Lt., MC (Assigned 23 September 1948)
Sullivan, Kathleen, CAF-3 (Stenographer) (Assigned 24 June 1948)

Virus and Rickettsial Section

Berge, Trygve O., Major, MSC, Chief
Burns, Kenneth F., Major, VC
Blender, John X., Captain, MC
Satterwhite, James P., Captain, MC (Assigned 18 February 1948)
Young, Irving, 1st Lt., MC (Assigned 23 October 1948)
Huiet, Ida D., P-2
Geib, Donna S., P-2 (Assigned 1 June 1948)

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